Sensitive 1,1-dicyanovinyl push-pull dye for primary amine sensing in solution by fluorescence

Lulu Bu, Maxime Rémond, Pauline Colinet, Erwan Jeanneau, Tangui Le Bahers, Frédéric Chaput, Chantal Andraud, Yann Bretonnière

Sensitive 1,1-dicyanovinyl push-pull dye for primary amine sensing in solution by fluorescence

Lulu Bu,[a,b] Maxime Rémond,[a] Pauline Colinet,[a] Erwan Jeanneau,[c] Tangui Le Bahers,[a] Frédéric Chaput,[a] Chantal Andraud,[a] Yann Bretonnière[a,*]

[a] Université de Lyon, École Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, CNRS UMR 5182, Laboratoire de Chimie, 46 allée d'Italie, F69364 Lyon, France

[b] School of Chemistry and Chemical Engineering, Huainan Normal University, Huainan, China

[c] Université de Lyon, Centre de Diffractométrie Henri Longchambon, Université Lyon I, 43 boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex (France)

* corresponding author: yann.bretonniere@ens-lyon.fr

Abstract

1,1-dicyanovinyl push-pull dye **PTTCN** undergoes rapid and reversible aza-Michael addition with volatile primary aliphatic amines in solution in DMSO, yielding a highly fluorescent imine derivative. This reaction is accompanied by a dramatic colour change as well as a strong increase in fluorescence (1200-fold increase) allowing sensitive turn-on detection of primary amine in solution. In the solid state, **PTTCN** is emissive and reaction with primary amines vapours results in a shift in fluorescence and a possible ratiometric detection of primary amine. The selectivity is excellent for primary amines over secondary, tertiary and aromatic amines as well as over thiols and fluorescence spectroscopy allow to discriminate between primary and other nitrogen nucleophiles such as hydrazine and ammonia. Finally, we show as proof-of-concept that the fluorescence turn-on response can be used in response to the vapours of volatile amines released during food spoilage.

Keywords: Primary amine; Fluorescence turn-on; Ratiometric; Fluorescent Thin Film

1. Introduction

Aliphatic primary amines are organic compounds omnipresent in the pharmaceutical, cosmetics or agrochemical industry, as well as in biology and it is often necessary to be able to accurately detect trace levels in real time and

in different matrices. Some natural primary amines of low-molecular weight, like histamine for instance, modulate essential metabolic and physiological functions in living organisms, but may also be of major health concern when present in food. They thus have been identified as important markers for evaluation of food quality or specific indicators for various diseases or health issues.[1-3]

For these reasons, many techniques have been developed so far for the qualitative and quantitative detection of amines, as diverse as high performance liquid chromatography, capillary electrophoresis, gas chromatography, or biosensor, to cite a few, each with its pro and cons.[4, 5] Of these, those based on chemical optical probes in general and photoluminescence (PL) in particular offer definite advantages in terms of rapidity, sensitivity with demonstrated limits of detection to nM, ease of handling and possibility to be used in solution or solid supports.[6, 7]

As recently summarized in excellent reviews, various strategies exploiting the basic or nucleophilic properties of amines have been reported to design chemical probes whose optical properties, whether absorption or PL, change upon exposure to amines.[6] [5, 7, 8]

For instance, direct addition of a nucleophilic amine to a chromophore's electron-withdrawing group or 1,2-addition to C=C double bond conjugated with this group heavily modifies the electron delocalisation within the dye molecule, therefore triggering consequent spectral changes (absorption and/or emission) and directly inducing a signalling response. Thus, reversible conversion of the trifluoroacetyl group into a hemiaminal function [9-11] or irreversible conversion of the tricyanovinyl group into the 1-amino-2,2-dicyanovinyl group [12] results in a decrease of the electron-withdrawing strength and consequently in a blue-shift in the absorbance or PL spectra. On the other hand, reversible addition of amine on aldehyde- [13-19] or ketone-containing [20] chromophores gave highly fluorescent imine or iminium derivatives with usually red-shifted optical properties.

Dicyanovinyl group is probably one of the simplest, yet powerful, electron-withdrawing groups for this effect. Aza-Michael nucleophilic addition of amine to the dicyanovinyl group is followed by the loss of a malononitrile molecule to yield an imine, leading to a large spectral-shift and most often a PL turn-on response. This was shown by Cheng and He who developed high sensitivity probes for primary amine vapour with polymers encompassing benzylidenemalononitrile units [21] and by Shi, Dong and co-workers who described the detection of amine vapours through formation of a bis-imine derivatives with a bis- dicyanovinyl based probes.[22] Previously, turn-on fluorogenic probe for detecting MDMA from ecstasy tablets [23] and dual-mode detection of aliphatic amines and hydrazine by PL [24] were also reported built upon a bis-diarylurea-

dicyanovinyl push-pull fluorophore and a dicyanovinyl-substituted oligothiophene derivative associated with zinc tetraphenylporphyrin, respectively. Mohr *et al.* also described a colorimetric read-out sensor based on push-pull azo dye and therefore working at wavelengths compatible with low-cost light sources and detectors.[25, 26] However in all these works, the end product was not characterized or simply postulated as being the 1,2 adduct.[26] Sathiyanarayanan *et al.* reported the design of a ratiometric PL sensor for primary amine based on a dicyanovinyl substituted phenanthridine derivatives and they clearly identified the end product as being the corresponding imine.[27] Finally, in 2020, Burn, Show and coworkers reported two dicyanovinyl-fluorene-benzothiadiazole-based fluorescent compounds, which showed rapid response to a wide range of amines. Primary amines react with the dicyanovinyl by aza-Michael (1,2) addition to give the corresponding imine, while PL quenching *via* photoinduced hole transfer was observed in the solid-state with other amines.[28]

It should be noted, however, that nucleophilic addition on dicyanovinyl based chromophores is not specific to primary amines. Hydrazine indeed yields hydrazone, a much weaker electron-withdrawing group than dicyanovinyl,[29] whereas thiols and cyanide give the 1,2-addition product. Here also the modification or the breaking of the dye π-conjugated system leads to considerable changes in the optical properties (blue shift and generally PL enhancement). Extremely sensitive and "selective" colorimetric and/or fluorescent sensors to hydrazine,[30-34] cyanide,[30, 35-42] and thiols [43, 44] [36, 45] are reported. In most cases, no mention is made of possible interferences from competitive amines.[25]

We have identified **2-((5'-(piperidin-1-yl)-[2,2'-bithiophen]-5-yl)methylene)malononitrile (PTTCN**, Scheme 1), a 1,1-dicyanovinyl push-pull dye, that undergoes rapid reaction with primary amine to yield fluorescent imine with emission intensities as high as 1200 times greater than the starting materials. We want to present here preliminary results about its use as fluorescent probe for primary amine detection in solution and in the solid state.

Compound **PTTCN** and other analogues, in which the 1,1-dicyanovinyl group is connected to a strong donor 2-(*N*-dialkyl)-amino- or 2-(*N*-diaryl)-amino- across a *2,2'*-bithiophene π-conjugated bridge conferring moderate aromatic stabilization to the donor-acceptor system and therefore a high degree of conjugation between the donor and acceptor end groups, were originally developed as highly polarizable chromophores for 2nd order nonlinear optics.[46, 47] [48, 49] The spectroscopic properties were later studied in detail and are characteristic of such donor-acceptor substituted heterocyclic compounds with a strong intramolecular charge transfer (ICT) absorption band in the visible region of the spectrum, and a weak red PL that decreases in intensity as the solvent

polarity increases.[50, 51] The PL also increased with the viscosity, making this compound and other analogues potential viscosity probes [52] and explaining the solid-state emission we observed. On the other hand, very few examples of imine derivatives built on the 2-(*N*-dialkylamino or diarylamino)-*2,2′*-bithiophene pattern are described,[53, 54] mostly chiral bichromophoric systems developed for CD exciton chirality studies or for second-order NLO applications. Bereva and coworkers, nevertheless, highlighted the intense blue-green PL of *N*-[[5'-(Dimethylamino)[2,2'-bithiophen]-5-yl]methylene]-cyclohexylamine and *N*-[[5'-(1-Pyrrolidinyl)[2,2'-bithiophen]-5-yl]methylene]-cyclohexylamine.[53]

2 Experimental

2.1 Materials and Methods

All solvents and reagents were commercially available and used without further purification. Column chromatography was carried out on silica gel (35-70 µm). ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on a Bruker Advance 400 spectrometer operating at 400.0 MHz for proton and 101.00 MHz for carbon. respectively. Chemical shifts are reported in parts per million (δ/ppm) relative to tetramethylsilane with the residual solvent peaks as internal standard (2.50 and 39.5 ppm for DMSO). High-resolution mass spectrometry (HRMS) were measured by ESI-TOF (Bruker Daltonics Micro TOF-Q II) at the *Centre Commun de Spectrométrie de Masse* (UCBL, Villeurbanne, France). UV/Vis absorption spectra were measured using a dual beam Jasco 670 spectrometer. Fluorescence spectra in diluted solution and in thin film were recorded on a Horiba Jobin-Yvon Fluorolog-3® spectrofluorimeter equipped with red-sensitive Hamamatsu R928 PMT. Spectra were reference corrected for both the excitation source light intensity variation (lamp and grating) and the emission spectral response (detector and grating).

2.2 Synthesis

2.2.1 Synthesis of 2-((5'-(piperidin-1-yl)-[2,2'-bithiophen]-5-yl)methylene)malononitrile (PTTCN)

In a 20 mL microwave tube, 5-formyl-5′-piperidino-2,2′-bithiophene (**1**) (300 mg, 1.08 mmol) and malononitrile (85 mg, 1.28 mmol) were dissolved in anhydrous EtOH (5 mL), followed by addition of one drop of piperidine. The tube was sealed and heated at 100°C by microwave irradiation for 1 h. After cooling down to room temperature, the solid was filtrated and purified by column chromatography on silica (eluent: CH_2Cl_2) to

afford **PTTCN** as a dark purple solid (244 mg, yield: 69%). ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.38 (s, 1H), 7.77 (d, ^{3}J = 4.3 Hz, 1H), 7.50 (d, ^{3}J = 4.3 Hz, 1H), 7.29 (d, ^{3}J = 4.3 Hz, 1H), 6.27(d, ^{3}J = 4.3 Hz, 1H), 3.29 (m, 4H), 1.61 (m, 4H), 1.82-1.43 (m,6H). ^{13}C NMR (101 MHz, DMSO-d_6,, ppm) δ 162.9, 151.4, 150.7, 143.6, 131.0, 129.8, 121.5, 117.5, 115.7, 114.9, 105.6, 68.6, 50.9, 24.5, 23.0. HR-MS (ESI-QTOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{16}N_3S_2$, 326.0786; found, 326.0770.

2.2.2 Synthesis of (E)-1-(5'-(piperidin-1-yl)-[2,2'-bithiophen]-5-yl)-N-propylmethanimine (PTT-imine)

Under at inert atmosphere, **1** (50.0 mg, 0.18 mmol) and n-propylamine (10.6 mg, 0.18 mmol) were dissolved in CH_2Cl_2 (2 mL) and 4 Å molecular sieve (MS) was added. The mixture was heated to reflux for 2 h. MS was filtered off. Evaporation under reduced pressure to dryness afforded **PTT-imine** as a yellow solid sufficiently pure to be used without further purification (57 mg, yield: 99%). ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 8.33 (s, 1H), 7.28 (d, J = 3.8 Hz, 1H), 7.06 (d, J = 4.0 Hz, 1H), 6.98 (d, J = 3.8 Hz, 1H), 6.08 (d, J = 4.0 Hz, 1H), 3.44 (t, J = 6.7 Hz, 2H), 3.23-2.99 (m, 4H), 1.80-1.35 (m, 8H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6, ppm) δ 159.5, 154.1, 140.7, 138.4, 132.1, 125.2, 120.8, 120.6, 104.6, 61.8, 51.3, 24.6, 23.7, 23.2, 11.7. HR-MS (ESI-QTOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{22}N_2S_2$, 319,1297; found, 319,1295.

2.2.3 Preparation of sol-gel film

Silica-based sol preparation from methyltriethoxysilane (MTEOS): In a 2 L flask, citric acid (3.96 g, 0.02 mole) was dissolved in DI water (396 mL, 22 moles). Under vigorous stirring, MTEOS (400 mL, 2 moles) was added to the aqueous solution. With the formation of an emulsion, the medium quickly became opaque and then increasingly transparent as the ethanol was released following the hydrolysis of MTEOS. The resulting mixture was heated to 45°C overnight under continuous slow stirring. The ethanol produced by the hydrolysis reaction of the alkoxysilane was subsequently removed from the reaction solution at reduced pressure. The silica-based sol was then extracted by adding ether which caused phase separation. The aqueous phase containing the citric acid was then separated. In order to completely remove citric acid from the sol, the ethereal phase was washed several times by adding water. The ether is then removed in vacuo and the resulting sol is dissolved in THF while controlling the solid content (54% by mass).

Preparation of doped sol-gel film: **PTTCN** (2.0 mg) dissolved in THF (1 mL) was added to the previously prepared MTEOS sol (2 g, 54 wt% in THF). Once the mixing has been carried out, part of the THF (1 mL) is removed under vacuum in order to obtain a viscous doped sol. The latter was spread evenly on a glass substrate by centrifugation at 2000 rpm. The sol-gel film was further dried by heating at 90°C for 1 h in an oven.

2.2.4 Preparation of PMMA thin film

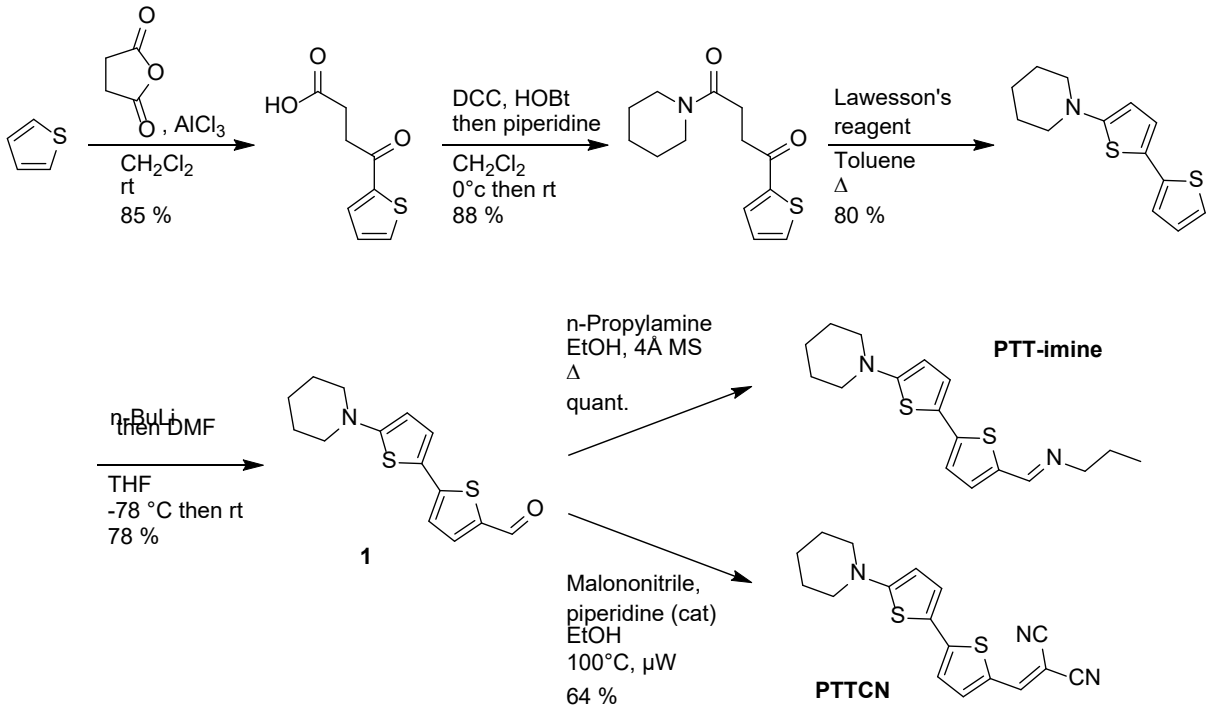
A solution of **PTTCN** and **PMMA** in dichloromethane (1 mg dye for 100 mg PMMA in 1 mL) was spread evenly on a glass surface by spin-coating at 2000 rpm using a Karl Suss CT 62 spin-coater.

2.3 Computational method

All calculations were performed using Gaussian package.[55]At the ground state, the structure of all the compounds was optimized at the DFT/6-311G(d) level of theory using hybrid functionals PBE0.[56] Bulk solvent effects (DMSO) were considered using continuum solvation model (C-PCM).[57, 58] For each compound, 10 vertical excitation energies and associated excited-state densities of interest were computed at the TD-DFT level with the same basis set and CAM-B3LYP functional.[59] The charge transfer transitions were characterized thank to the DCT index developed by Le Bahers et al. using the cube files generated by the cubegen utility provided by the Gaussian package.[60] To simulate the colour, the trichromatic components were obtained from the transmission spectra through a code developed in our group and converted into RGB ones.

3 Results and discussion

3.1 PTTCN and PTT-imine synthesis and characterization



Scheme 1. Synthesis of **PTTCN** and **PTT-imine**.

PTTCN was obtained as a dark violet powder from malononitrile and aldehyde **1** by Knovenagel condensation. **1** was prepared in five steps from thiophene, succinic anhydride and piperidine by construction of the second thiophene ring by a combination of Friedel-Craft and Lawesson reactions,[61] followed by formylation by α-lithiation and reaction with DMF (Scheme 1). This approach was preferred to classical bithiophene synthesis using palladium catalysed coupling reaction as it gives higher yield. **PTT-imine** was in turn easily obtained from **1** by reaction with n-propylamine in presence of 4 Å MS.

Crystals of **PTTCN**, suitable for X-ray diffraction, were grown by slow diffusion of EtOH in concentrated dye solution in $CHCl_3$. Crystallographic data, basic structural parameters and the X-ray crystal structure are given in SI. The latter showed a quasi-planar π-delocalized system with twenty-two atoms forming the molecular mean plane (maximum deviation of 0.114 Å). Only the dicyanovinylene group is slightly twisted forming a 5.7° angle with the bis-thiophene entity. This is indicative of a full conjugation between the donor and the acceptor end of the push-pull molecule.

In solution, the electronic absorption spectrum of **PTTCN** is characteristic of intramolecular charge transfer (ICT) transitions in push-pull dipolar molecules with a broad absorption in the visible (λ_{abs} = 575 nm in DMSO, in good agreement with the calculated value of 509 nm, cam-B3LYP/6-311G(d)/PCM(DMSO), large molar

absorption coefficient values, and a small bathochromic shift in the maximum as the solvent polarity increases (Figure SI 2). Upon excitation in the main absorption band PL is observed, whose peak maximum is gradually red-shifted and decreases in intensity as the solvent polarity increases from toluene (λ_{em} = 640 nm, φ = 18 %) to DMSO where it almost vanishes (λ_{em} = 695 nm, φ < 1 %). **PTTCN** also showed interesting far-red emission in the solid state (λ_{em} = 665 nm, φ = 24 %) easily visualized under illumination of a handheld UV lamp at 365 nm.

3.2 Sensing properties in solution

The reaction **PTTCN** with primary amine was first investigated by ^{1}H-NMR. Stepwise addition of n-propylamine to a solution of **PTTCN** in -DMSO-d_6 immediately yielded the formation of a single new product that concurs with colour change from violet to yellow along with appearing of an intense green PL that can be seen under UV light. The ^{1}H-NMR spectrum (Figure 1) of the new product displayed similar splitting patterns but significant upfield shifts for aromatic protons and the persistence of a vinylidene singlet integrating for one proton at 8.33 ppm. This is in agreement with an imine structure in which the push-pull character is preserved but a weaker electron-acceptor group is present. A simple nucleophilic reaction at the Cβ position, as assumed by Finšgar *et al.* for similar dicyanovinyl azobenzene dyes [26] or as is observed with thiols [25] and the cyanide anion,[35, 42] would lead to a drastic upfield shift of the proton originally at the β-position as well as those of the closest thiophene ring due to a disruption of the π-conjugation from the donor to the acceptor end. The imine structure was further confirmed by mass spectrometry and the independent synthesis of **PTT-imine** from aldehyde **1** and n-propylamine (Scheme 1), whose ^{1}H and ^{13}C NMR spectra (Figure SI 15 and SI 16) perfectly match those of the new product.

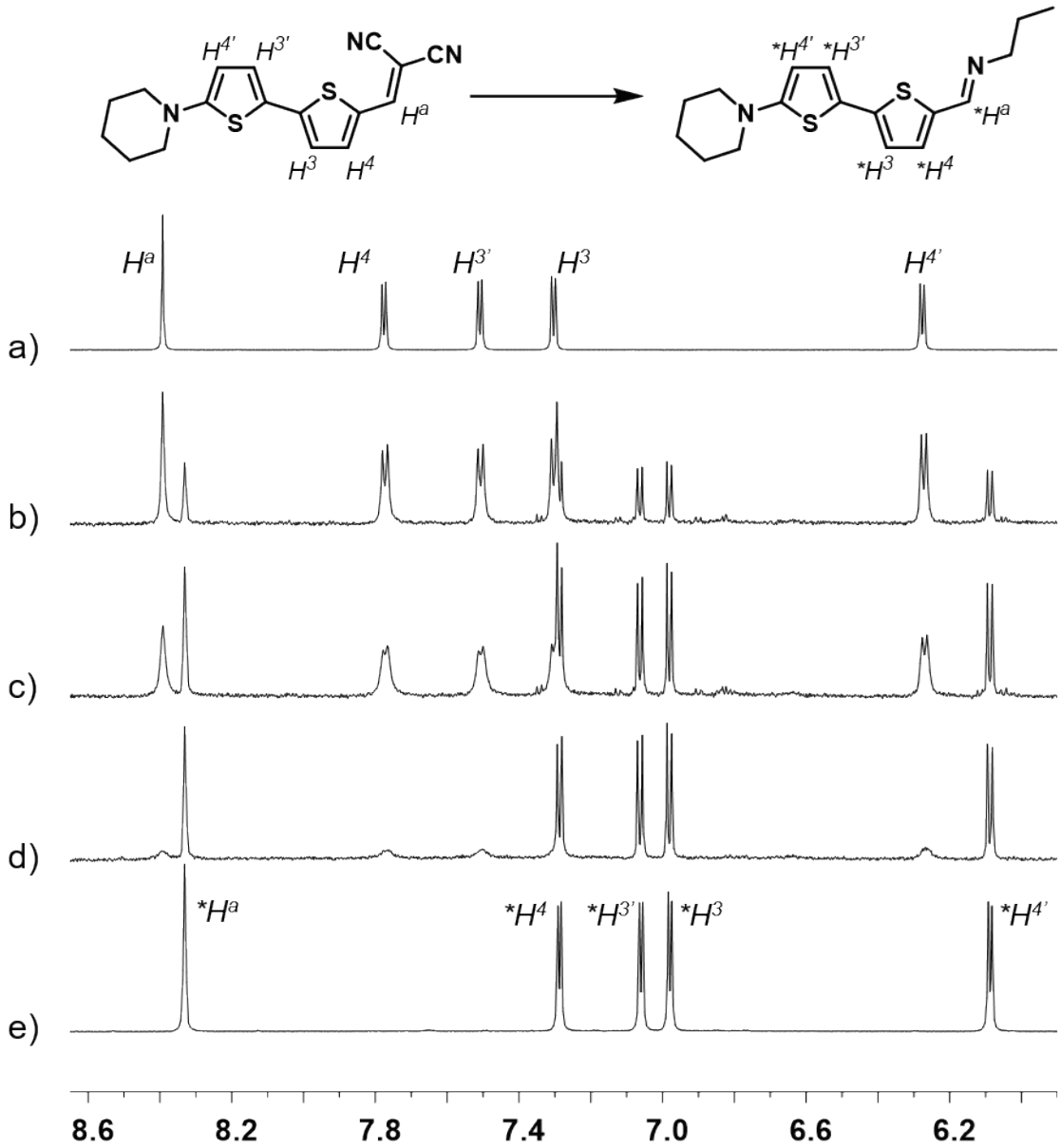


Figure 1. Aromatic region of the ^{1}H-NMR spectra (300 MHz, DMSO-d6) of **PTTCN** prior (a) and immediately after addition of 0.6 equiv. (b), 1.2 equiv. (c), 2.5 equiv. (d), and 4 equiv. (e) of n-propylamine.

More interestingly, in the case of a volatile amine having low boiling point such as n-propylamine, ^{1}H NMR also evidences the reverse transformation of imine to the initial dicyanovinyl (Figure SI 4). Removal of the excess amine by evaporation under vacuum induced a colour change back to the original purple of dye **PTTCN** as well as a disappearance of the PL and the reappearing of the NMR signal of **PTTCN**. Note, however, that addition of excess malononitrile to a solution of **PTT-imine** in DMSO-d_6 immediately resulted in a colour change from yellow-green to dark red then purple, but the signals obtained are broad and the reformation of **PTTCN** could not be unambiguously demonstrated.

Spectroscopic studies also evidenced the interest of **PTTCN** as a primary amine fluorescent probe. The changes in the absorption and PL spectra on addition of n-octylamine (or n-propylamine) to solution of **PTTCN** in DMSO (10 µM) are shown in Figure 2. With increasing amount of amine added, the characteristic ICT band at 575 nm disappeared while the colour of the dye solution turns yellow with the concomitant appearing of a new peak with a maximum absorbance at 412 nm. The isobestic point at 462 nm indicates that a single chemical reaction takes place. Kinetic studies revealed that the reaction is first order with respect to both reactants primary amine and **PTTCN** (Figures SI 5 and SI 6).

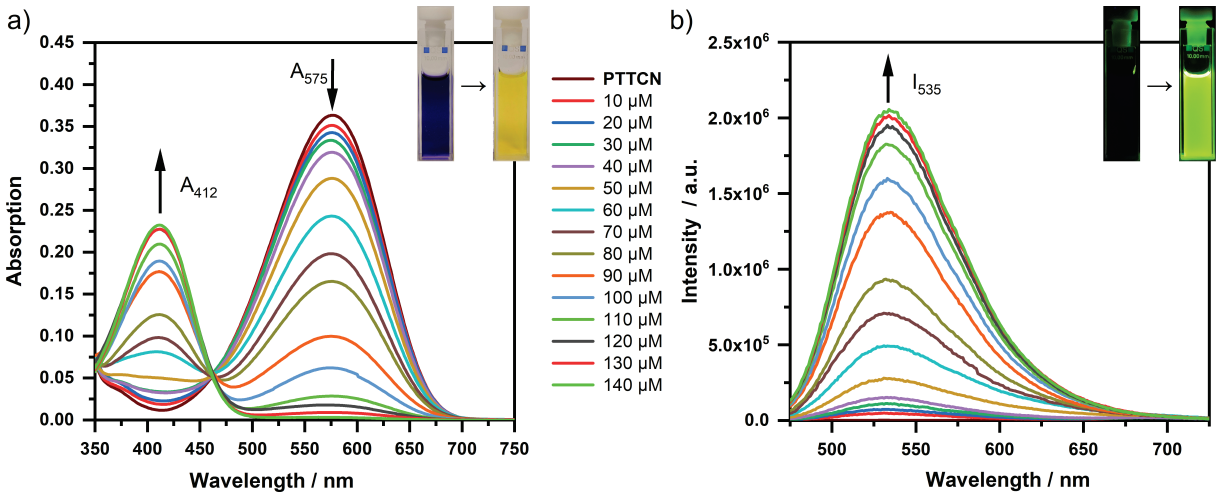


Figure 2. Spectral changes for a solution of **PTTCN** (10 µM) in DMSO immediately after addition of increasing amount of n-octylamine (0 to 140 µM): (a) absorption and (b) emission spectra (λex = 462 nm, isobestic point of the absorption spectra). Inset: solution of **PTTCN** without (left) and with (right) excess octylamine (140 µM), daylight and under UV-light (365 nm).

At the same time, an intense emission appeared at 535 nm that steadily increased while adding amine. At this high dye concentration (10 µM) the reaction is fast and the fluorescence is therefore seen immediately. The quantum yield of the newly formed product reaches unity (φ = 0.96) whereas **PTTCN** itself is barely emissive in DMSO, a "turn-on" PL response (1160-fold emission intensity enhancement, Figure 2b) is then achieved. As shown is Figure SI 7, a theoretical limit of detection of n-octylamine was estimated to be as low as 60 nM for a dye concentration of 5 µM. Note however that for such low concentration in amine, the reaction is much slower and the measure has to be performed 15 hours after amine addition.

The dramatic colour change observed when switching from dicyonovinyl to imine is clearly explained by a

decrease in intramolecular charge transfer and in the push-pull character. This is confirmed by DFT and TD-DFT calculations on **PTTCN** and **PTT-imine**.

Calculations reproduced the modification of the absorption properties towards the blue range could also be reproduced computationally (Figure 3-a) with a shift of the wavelength of maximum absorption from 509 nm (0.28 eV difference compared to experimental value) for **PTTCN** to 383 nm (0.15 eV difference compared to experimental value) for the **PTT-imine**. A fluorescence maximum at 505 nm is computed in good agreement with what is observed (0.14 eV difference). The computed distance characteristic of the charge transfers under absorption decreases from 3.41 A to 2.95 A from **PTTCN** to **PTT-imine** as illustrated on Figure 3-b, in good agreement with a less efficient charge transfer.

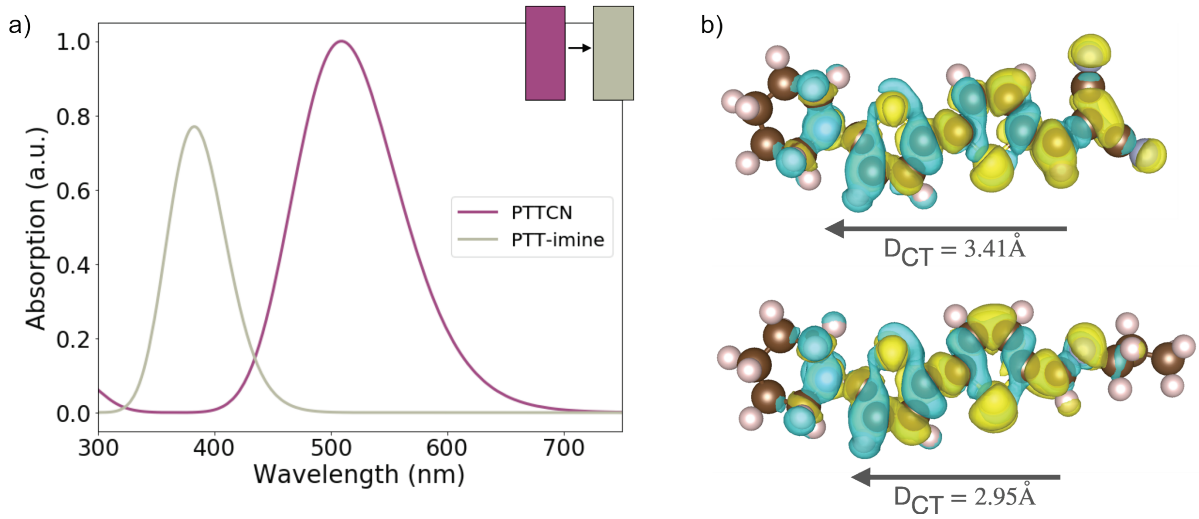


Figure 3. b) Simulated absorption spectra (at CAM-B3LYP/6-311G(d) level of theory) of the two molecules in DMSO along with their simulated colour (top insert). b) Representation of the difference between excited- and ground-state electron densities (cyan, density increase upon transition; yellow, density decrease; isovalue of .0005) as well as the CT distance (in grey) for PTTCN (top) and PTT-imine (bottom) molecules. Carbon, hydrogen, sulfur and nitrogen atoms are depicted respectively by brown, white, yellow and blue spheres.

3.3 Selectivity

Qualitative PL-sensing studies were performed with various aliphatic amines (ammonia, putrescine, tyramine, cadaverine, propylamine, histamine, tryptamine, spermidine, 1,3-diamine, diethylamine, diisopropylamine, trimethylamine), aromatic amine (aniline, p-anisidine, *N,N*-dimethyl-p-phenylenediamine) and other nucleophile (ethanethiol, hydrazine). Addition of secondary amine (diethylamine, diisopropylamine), tertiary amine

(trimethylamine) or thiol (ethanethiol) did not induce any colour change nor any significant change in the PL spectra of **PTTCN** in DMSO solution (Figure 4-a). The same applies when an aromatic amine is added, including electron enriched p-anisidine and N,N-dimethyl-p-phenylenediamine. However, when a primary amine (here n-propylamine, 50 equiv.) was added after addition of 50 equivalent of diethylamine, trimethylamine, or thiol, the colour immediately changed and the strong PL characteristic of the imine formation is immediately observed highlighting the selectivity for the primary amine and the absence of interference from secondary analytes (Figure 4-b).

On the other hands, **PTTCN** has fast PL-response to ammonia and hydrazine compounds (hydrazine or dimethylhydrazine). However, the new products obtained in these cases display slightly blue-shifted absorption and emission compared to the imine for hydrazine and red-shifted for other analytes, and are less emissive (Figure 5, Table 1), allowing to distinguish them.

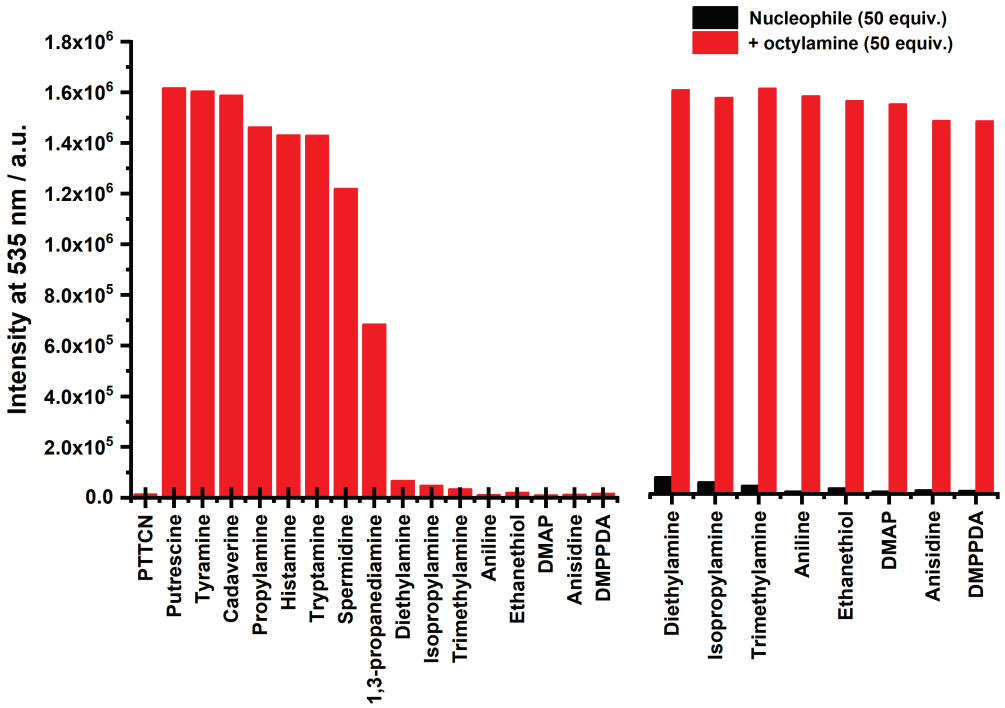


Figure 4. PL intensity at 535 nm (λ_{exc} = 460 nm) of 10 µM **PTTCN** solution in DMSO with: a) different amines and nucleophiles (50 equiv); b) different amines and nucleophiles (50 equiv) then n-octylamine (50 equiv), in black the initial PL intensity before adding n-octylamine.

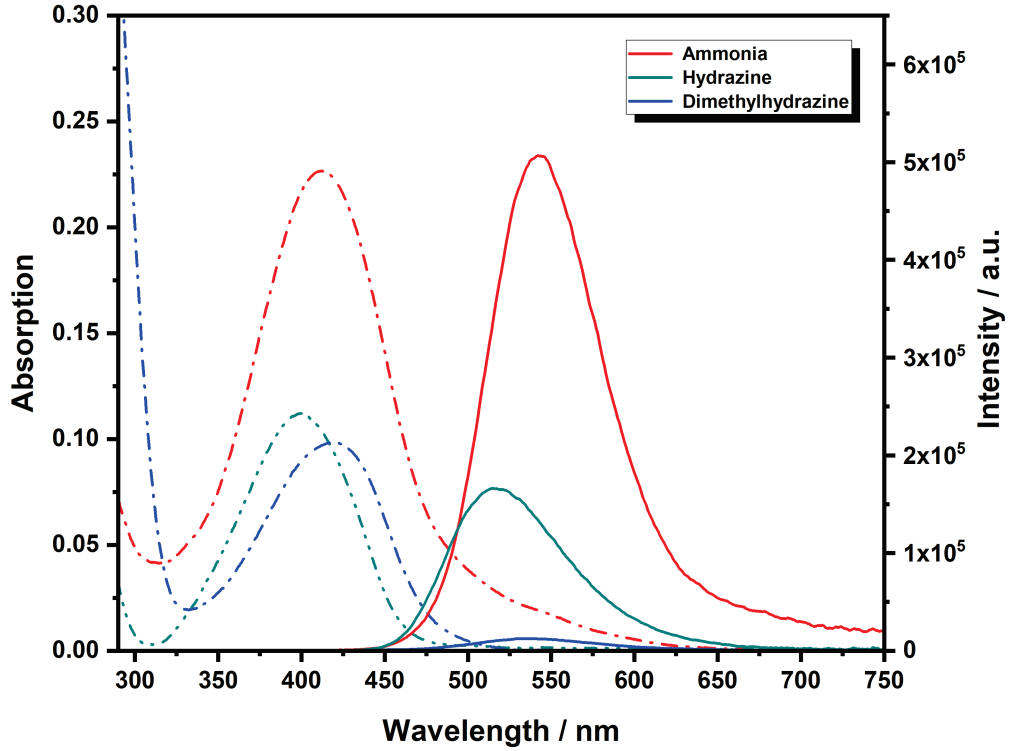


Figure 5. Absorption (dotted lines) and emission spectra (solid lines) of **PTTCN** upon addition of excess of ammonia, hydrazine or dimethylhydrazine (10 µM in DMSO, λ_{exc} = 400 nm).

Table 1. Spectroscopic data for **PTTCN** and for **PTTCN** with addition of various N nucleophiles.

	PTTCN			+ n-propylamine			+ Ammonia			+ Hydrazine			+ N,N-dimethylhydr.		
	λ_{abs} (nm)	λ_{em} (nm)	Φ	λ_{abs} (nm)	λ_{em} (nm)	Φ	λ_{abs} (nm)	λ_{em} (nm)	Φ	λ_{abs} (nm)	λ_{em} (nm)	Φ	λ_{abs} (nm)	λ_{em} (nm)	Φ
DMSO	575	695	< 0.01	412	535	0.96	413	543	0.58	400	516	0.30	419	541	0.03
Film (sol-gel)	566	662	23	403	506	0.43	410	543	0.26	398	514	0.15	418	535	0.01
Film (PMMA)	553	656	13	404	499	0.57	nd	nd	nd	nd	nd	nd	nd	nd	nd

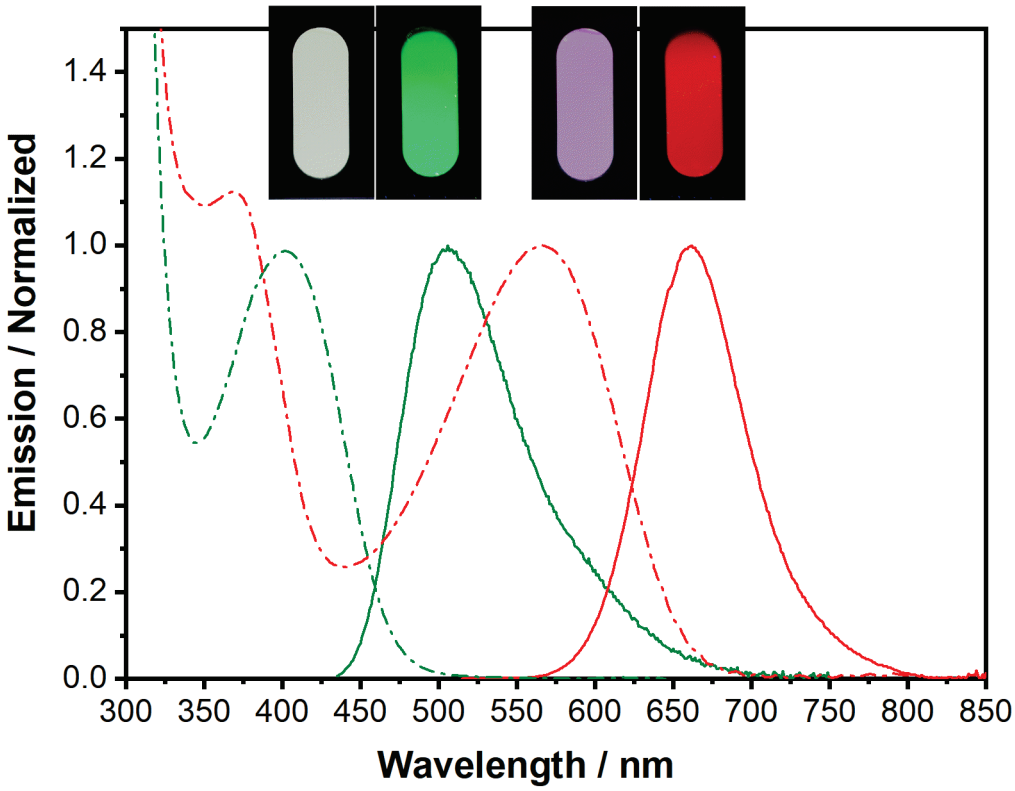
3.4 Primary amine vapour sensing in thin-film

Having established **PTTCN** as a robust PL turn-on and reversible probe to detect primary amines in solution, we then investigated primary amine vapour detection by thin films of the sensing material. For this purpose, optically thin films of **PTTCN** in sol/gel or PMMA were obtained on glass substrate by spin-coating. The violet films displayed large absorption band at 566 nm and 553 nm respectively and showed strong far-red PL upon excitation at the absorption peak (λ_{em} = 661 nm / φ = 23% and λ_{em} =656 nm / φ = 13 %, for sol-gel and PMMA films resp., Table 1). We then choose n-propylamine as an example for its high volatility, to test the film upon exposure to amine vapours. Exposing the sol-gel films to an atmosphere saturated with n-propylamine vapours

induced colour change from violet to almost colourless (λ_{max} = 402 nm) with complete transformation in less than 5 minutes. For PMMA films, the same colour change is observed (λ_{max} = 403 nm) upon exposure to amine vapour, but total conversion takes more than 20 min. The faster kinetic observed in sol-gel is probably due to the more porous material in this case and therefore the greater accessibility of the dye. In the same time, for both kind of films, a significant variation of emission from red to green was observed by naked eye when the films are illuminated under 365 nm UV-light (Figure 6, see also SI). Upon excitation in the absorption peak strong green PL signals were observed for the sol-gel and the PMMA films after exposure with amine, with maxima at 508 nm and 497 nm respectively. Good emission quantum yields were measured, respectively Φ = 57 % and Φ = 43 %. A ratiometric PL response to primary amine vapour is therefore possible when choosing the excitation wavelength at 455 nm, wavelength at which the absorption of both **PTTCN** and imine are identical.

Similar colour changes were observed with exposure to ammonia or dimethylhydrazine vapours (Figure SI 8). PLQY quantum yields in solid reflect what was observed in solution with weaker values for ammonia and dimethylhydrazine than for n-propylamine. Always in agreement to what was observed in solution, no fluorometric nor colourmetric responses were observed, even after 1 h, when the films were exposed to vapours of secondary amine, tertiary amine, ether, ester, amide, alcohol, or thiol.

Unfortunately, the reversibility highlighted in solution could not easily be reproduced. Vacuum pumping of the film exposed to propylamine vapour partially converts the film to its original state, but pumping for too long results in severe flaking-off the films. The same observation was made when fuming malononitrile vapours after amine exposure. The colour of the films reverted back to purple and the original emission partially recovered, but the sample were damaged.



304

305 **Figure 6.** Normalized absorption (dotted line) and PL spectra (solid line) of **PTTCN** sol-gel film before (red) and
306 after (green) exposure to n-propylamine vapour. Inset: the photography showed the film mounted in the support
307 before (right) and after (left) exposure to n-propylamine of **PTTCN** in the absence (black) and presence (red) of
308 saturated propylamine vapor under daylight or UV-light (365 nm).

309

310 **3.5 Tentative of monitoring seafood freshness**

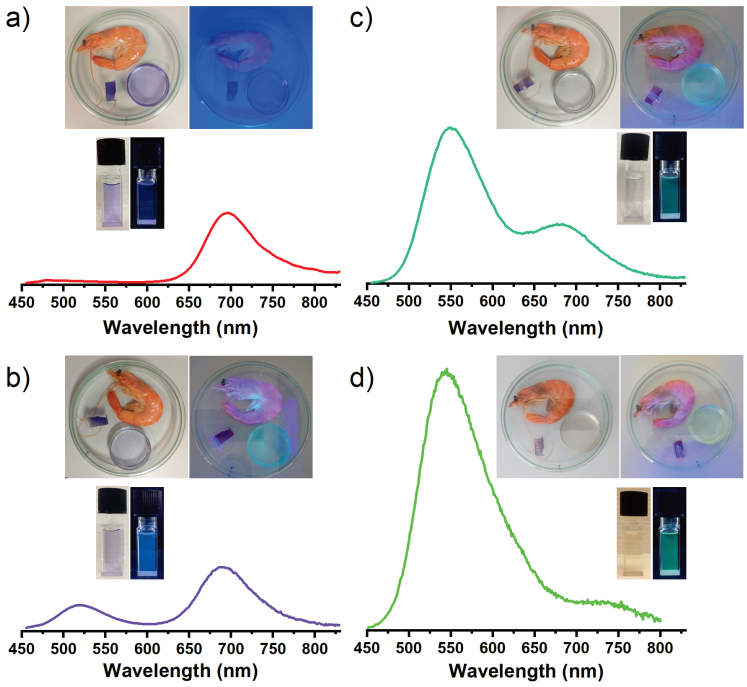


Figure 7. Attempt to control shrimp freshness, followed by a week: a) initial situation, b) after 24 hours, c) after 72 hours, d) after 150 hours. For each time: picture of a solution (20 µM in DMSO in the small crystallizer) and of a sol-gel film on a glass substrate, placed inside the box with the whole animal, under daylight (left) and under handheld UV light (right); picture of the spectroscopic cell used for recording the fluorescence under daylight (left) and under 365nm UV-light of a portable lamp (right); and emission spectra recorded in the spectrofluorimeter – λ_{exc} = 430 nm.

Various volatile amine/ammonia vapours are generated during food spoilage. Using shrimps as an example, we tested whether **PTTCN** and the prepared materials can be used as fluorescent probe for monitoring seafood freshness. To do so, sol-gel spin-coated film as well as a DMSO solution of **PTTCN** (20 µM) were placed inside a packing box in which a shrimp was stored at room temperature for 1 week. A picture of the whole was taken every 24 hours and the emission spectra of the solution and films (λ_{exc} = 430 nm) were also recorded. These data are summarised in Figure 7. Initially, only **PTTCN** emission was observed both in film and in solution (weak emission in concentrated solution, Figure 7a). Changes can be directly observed by naked eye in the solution sample. After 24 h, while the solution was still purple, indicating that **PTTCN** was still the main species in solution, a blue fluorescence emanated from the solution when viewed under 365 nm UV-light. The corresponding emission spectrum (Figure 7b) showed the appearing of a new emission band at 540 nm, in addition to the main **PTTCN** emission. This new band strongly increased in intensity after 48 and then 72 hours

(Figure 7b and 7c), as the solution lost its purple colour to grey, and then became almost the only detectable emission after one week when the solution turned yellow (Figure 7d).

Unfortunately, during the same period of time, the changes to the film are not as visually spectacular. The overall colour does not change and remains purple, albeit slightly faded, as does the fluorescence when viewed under 365 nm UV-light, dominated by the intense red emission of **PTTCN**. It is only when the emission spectra are recorded with a spectrofluorimeter, that a change is clearly seen with the appearing of a new band at 540 nm in addition to the (intense) fluorescence of **PTTCN** (Fig. SI-12) when exciting at a wavelength close to the imine absorption maximum (λ_{exc} = 430 nm). When PMMA films are used instead, no change both in absorption and fluorescence spectra are observed. This could originate from the weak concentration of amine in the atmosphere of the box reducing the kinetic of the reaction that is already slower in PMMA than in sol-gel film (see above).

While these film results confirm the previous observations and the formation of new emissive species, they also reveal the limitation of the system. The detection of volatile amines during food spoilage could not be easily followed with the naked eye, although it could be possible with the use of an appropriate measuring instrument.

Conclusion

In solution in DMSO or embedded in PMMA or sol-gel films, the dicyanovinyl compound **PTTCN** reacts with strong nucleophilic nitrogen-containing analytes and especially primary aliphatic amine in a transformation dicyanovinyl $\rightarrow$ imine, that is reversible in nature. This transformation results in significant changes in the optical properties, particularly fluorescence. **PTTCN** is only slightly emissive but displays a large emission in the far-red in the solid-state, while the imine compounds formed by reaction with primary amines, on the other hand, are highly emissive in both solution and in the solid state. In consequence, **PTTCN** can serve as a very sensitive turn-on probe to detect primary amines in solution with a 1200-fold enhancement of the fluorescence intensity during the reaction of **PTTCN** with n-propylamine. Sensitivity in the nanomolar range by fluorescence monitoring was achieved and an excellent selectivity for primary amines over other amine nucleophiles was evidenced both in solution and in the solid-state. We have also shown that monitoring of seafood spoilage is possible with colour changes in response to the vapours released during shrimp spoilage, visible to naked-eye accompanied by spectacular fluorescence enhancement over time in solution. Spin-coated films can also serve as probe, highlighting the potential of **PTTCN** for low-cost amine probes, even though the response is less obvious and only visible by instrumental recording.

Declaration of Competing Interest

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.

Acknowledgements

L.B. thank the thanks the Chinese scholarship council and ENS de Lyon for a PhD grant.

Appendix A. Supplementary data

The following is Supplementary data to this article: Experimental details, additional figures, films and characterizations.

References

[1] Y. Özogul, F. Özogul, Chapter 1: Biogenic amines formation, toxicity, regulations in food, in Biogenic amines in food: Analysis, occurrence and toxicity, The Royal Society of Chemistry, 2020, pp. 1-17, https://doi.org/10.1039/9781788015813-00001.

[2] C. Ruiz-Capillas, A. M. Herrero, Impact of biogenic amines on food quality and safety, Foods 8 (2019) 62, https://doi.org/10.3390/foods8020062

[3] D. Doeun, M. Davaatseren, M.-S. Chung, Biogenic amines in foods, Food Sci Biotechnol 26 (2017) 1463-1474, https://doi.org/10.1007/s10068-017-0239-3.

[4] A. Önal, A review: Current analytical methods for the determination of biogenic amines in foods, Food Chem. 103 (2007) 1475-1486, https://doi.org/10.1016/j.foodchem.2006.08.028.

[5] S. K. Kannan, B. Ambrose, S. Sudalaimani, M. Pandiaraj, K. Giribabu, M. Kathiresan, A review on chemical and electrochemical methodologies for the sensing of biogenic amines, Anal. Methods 12 (2020) 3438-3453, https://doi.org/10.1039/d0ay00358a.

[6] J. L. Klockow, K. S. Hettie, T. E. Glass, Fluorescent sensors for amines, in: Atwood JL, Comprehensive supramolecular chemistry II, Elsevier, Oxford, 2017, pp. 447-467, https://doi.org/10.1016/b978-0-12-409547-2.12630-7.

[7] A. I. Danchuk, N. S. Komova, S. N. Mobarez, S. Y. Doronin, N. A. Burmistrova, A. V. Markin, A. Duerkop, Optical sensors for determination of biogenic amines in food, Anal. Bioanal. Chem. 412 (2020) 4023-4036, https://doi.org/10.1007/s00216-020-02675-9.

[8] N. Kaur, S. Chopra, G. Singh, P. Raj, A. Bhasin, S. K. Sahoo, A. Kuwar, N. Singh, Chemosensors for biogenic amines and biothiols, J. Mater. Chem. B 6 (2018) 4872-4902, https://doi.org/10.1039/c8tb00732b.

[9] G. J. Mohr, C. Demuth, U. E. Spichiger-Keller, Application of chromogenic and fluorogenic reactands in the optical sensing of dissolved aliphatic amines, Anal. Chem. 70 (1998) 3868-3873, https://doi.org/10.1021/ac980279q.

[10] S. Reinert, G. J. Mohr, Chemosensor for the optical detection of aliphatic amines and diamines, Chem Commun (2008) 2272-2274, https://doi.org/10.1039/b717796h.

[11] S. Körsten, G. J. Mohr, Star-shaped tripodal chemosensors for the detection of aliphatic amines, Chem. Eur. J. 17 (2011) 969-975, https://doi.org/10.1002/chem.201000787.

[12] G. J. Mohr, A tricyanovinyl azobenzene dye used for the optical detection of amines via a chemical

reaction in polymer layers, Dyes Pigm. 62 (2004) 77-81, https://doi.org/10.1016/j.dyepig.2003.11.011.
[13] K. Secor, J. Plante, C. Avetta, T. Glass, Fluorescent sensors for diamines, J. Mater. Chem. 15 (2005) 4073-4077, https://doi.org/10.1039/b503269e.
[14] E. K. Feuster, T. E. Glass, Detection of amines and unprotected amino acids in aqueous conditions by formation of highly fluorescent iminium ions, J. Am. Chem. Soc. 125 (2003) 16174-16175, https://doi.org/10.1021/ja036434m.
[15] B. Lee, R. Scopelliti, K. Severin, A molecular probe for the optical detection of biogenic amines, Chem Commun 47 (2011) 9639-9641, https://doi.org/10.1039/c1cc13604f.
[16] C. Patze, K. Broedner, F. Rominger, O. Trapp, U. H. F. Bunz, Aldehyde cruciforms: Dosimeters for primary and secondary amines, Chem. Eur. J. 17 (2011) 13720-13725, https://doi.org/10.1002/chem.201101871.
[17] J. Freudenberg, J. Kumpf, V. Schäfer, E. Sauter, S. J. Wörner, K. Brödner, A. Dreuw, U. H. F. Bunz, Water-soluble cruciforms and distyrylbenzenes: Synthesis, characterization, and pH-dependent amine-sensing properties, J. Org. Chem. 78 (2013) 4949-4959, https://doi.org/10.1021/jo400576y.
[18] J. Kumpf, J. Freudenberg, S. T. Schwaebel, U. H. F. Bunz, Amine sensing with distyrylbenzenes and their hexamethylene-linked polymers: Spraying them on, Macromolecules 47 (2014) 2569-2573, https://doi.org/10.1021/ma500486u.
[19] J. L. Klockow, T. E. Glass, Development of a fluorescent chemosensor for the detection of kynurenine, Org. Lett. 15 (2013) 235-237, https://doi.org/10.1021/ol303025m.
[20] V. Valderrey, A. Bonasera, S. Fredrich, S. Hecht, Light-activated sensitive probes for amine detection, Angew. Chem. Int. Ed. 56 (2017) 1914-1918, https://doi.org/10.1002/anie.201609989.
[21] L. Shi, Y. Fu, C. He, D. Zhu, Y. Gao, Y. Wang, Q. He, H. Cao, J. Cheng, A mild and catalyst-free conversion of solid phase benzylidenemalononitrile/benzylidenemalonate to N-benzylidene-amine and its application for fluorescence detection of primary alkyl amine vapor, Chem Commun 50 (2014) 872-874, https://doi.org/10.1039/c3cc48299e.
[22] L. Kong, Y. Zhang, H. Mao, X. Pan, Y. Tian, Z. Tian, X. Zeng, J. Shi, B. Tong, Y. Dong, Dimalononitrile-containing probe based on aggregation-enhanced emission features for the multi-mode fluorescence detection of volatile amines, Faraday Discuss. 196 (2017) 101-111, https://doi.org/10.1039/c6fd00178e.
[23] D. Moreno, B. D. d. Greñu, B. García, S. Ibeas, T. Torroba, A turn-on fluorogenic probe for detection of MDMA from ecstasy tablets, Chem Commun 48 (2012) 2994-2996, https://doi.org/10.1039/c2cc17823k.
[24] T. Liu, L. Yang, W. Feng, K. Liu, Q. Ran, W. Wang, Q. Liu, H. Peng, L. Ding, Y. Fang, Dual-mode photonic sensor array for detecting and discriminating hydrazine and aliphatic amines, ACS Appl. Mater. Inter. 12 (2020) 11084-11093, https://doi.org/10.1021/acsami.0c00568.
[25] T. Mastnak, A. Lobnik, G. J. Mohr, M. Turel, Design and characterization of dicyanovinyl reactive dyes for the colorimetric detection of thiols and biogenic amines, Sensors 18 (2018) 814, https://doi.org/10.3390/s18030814.
[26] T. Mastnak, A. Lobnik, G. J. Mohr, M. Finšgar, Indicator layers based on ethylene-vinyl acetate copolymer (EVA) and dicyanovinyl azobenzene dyes for fast and selective evaluation of vaporous biogenic amines, Sensors 18 (2018) 4361, https://doi.org/10.3390/s18124361.
[27] M. Saravanakumar, B. Umamahesh, R. Selvakumar, J. Dhanapal, S. K. Ashok kumar, K. I. Sathiyanarayanan, A colorimetric and ratiometric fluorescent sensor for biogenic primary amines based on dicyanovinyl substituted phenanthridine conjugated probe, Dyes Pigm. 178 (2020) 108346, https://doi.org/https://doi.org/10.1016/j.dyepig.2020.108346.
[28] G. Zhang, A. S. Loch, J. C. M. Kistemaker, P. L. Burn, P. E. Shaw, Dicyanovinyl-based fluorescent sensors for dual mechanism amine sensing, J. Mater. Chem. C 8 (2020) 13723-13732,

https://doi.org/http://doi.org/10.1039/d0tc03974h.
[29] B. Roy, S. Bandyopadhyay, The design strategies and mechanisms of fluorogenic and chromogenic probes for the detection of hydrazine, Anal. Methods 10 (2018) 1117-1139, https://doi.org/10.1039/c7ay02866k.
[30] Y. Tan, J. Yu, J. Gao, Y. Cui, Y. Yang, G. Qian, A new fluorescent and colorimetric probe for trace hydrazine with a wide detection range in aqueous solution, Dyes Pigm. 99 (2013) 966-971, https://doi.org/10.1016/j.dyepig.2013.08.008.
[31] M. Sun, J. Guo, Q. Yang, N. Xiao, Y. Li, A new fluorescent and colorimetric sensor for hydrazine and its application in biological systems, J. Mater. Chem. B 2 (2014) 1846-1851, https://doi.org/10.1039/c3tb21753a.
[32] J. Fan, W. Sun, M. Hu, J. Cao, G. Cheng, H. Dong, K. Song, Y. Liu, S. Sun, X. Peng, An ICT-based ratiometric probe for hydrazine and its application in live cells, Chem Commun 48 (2012) 8117-8119, https://doi.org/10.1039/c2cc34168a.
[33] Y. Zhang, L. Yuan, S. Jia, X. Liu, J. Zhao, G. Yin, Dicyanovinyl substituted push–pull chromophores: effects of central C=C/phenyl spacers, crystal structures and application in hydrazine sensing, Phys. Chem. Chem. Phys. 21 (2019) 3218-3226, https://doi.org/10.1039/c8cp07127f.
[34] B. Chen, X. Sun, X. Li, H. Ågren, Y. Xie, TICT based fluorescence "turn-on" hydrazine probes, Sens. Actuator B-Chem. 199 (2014) 93-100, https://doi.org/10.1016/j.snb.2014.03.087.
[35] X. Cheng, Y. Zhou, J. Qin, Z. Li, Reaction-based colorimetric cyanide chemosensors: Rapid naked-eye detection and high selectivity, ACS Appl. Mater. Inter. 4 (2012) 2133-2138, https://doi.org/10.1021/am3001083.
[36] J. w. Jeong, S. Angupillai, I. J. Kim, J. Jeong, H.-S. Kim, H.-S. So, Y.-A. Son, Michael addition-based colorimetric and fluorescence chemodosimeters for the nanomolar-level tracking of cyanide ions in aqueous-organic media, Sens. Actuator B-Chem. 237 (2016) 341-349, https://doi.org/10.1016/j.snb.2016.06.107.
[37] Q. Zhang, J. Zhang, H. Zuo, C. Wang, Y. Shen, A novel colorimetric and fluorescent sensor for cyanide anions detection based on triphenylamine and benzothiadiazole, Tetrahedron 72 (2016) 1244-1248, https://doi.org/10.1016/j.tet.2016.01.019.
[38] A. Panja, K. Ghosh, Pyridylazo derivatives with dicyanovinyl appendage in selective Sensing of CN^- in sol-gel medium, ChemistrySelect 3 (2018) 1809-1814, https://doi.org/10.1002/slct.201702839.
[39] L. Wang, L. Li, D. Cao, A BODIPY-based dye with red fluorescence in solid state and used as a fluorescent and colorimetric probe for highly selective detection of cyanide, Sens. Actuator B-Chem. 239 (2017) 1307-1317, https://doi.org/10.1016/j.snb.2016.09.112.
[40] Y.-D. Lin, Y.-S. Pen, W. Su, K.-L. Liau, Y.-S. Wen, C.-H. Tu, C.-H. Sun, T. J. Chow, Reaction-based colorimetric and ratiometric fluorescence sensor for detection of cyanide in aqueous media, Chem. Asian J. 7 (2012) 2864-2871, https://doi.org/10.1002/asia.201200578.
[41] J. Orrego-Hernández, J. Portilla, Synthesis of dicyanovinyl-substituted 1-(2-pyridyl)pyrazoles: Design of a fluorescent chemosensor for selective recognition of cyanide, J. Org. Chem. 82 (2017) 13376-13385, https://doi.org/10.1021/acs.joc.7b02460.
[42] E. Thanayupong, K. Suttisintong, M. Sukwattanasinitt, N. Niamnont, Turn-on fluorescent sensor for the detection of cyanide based on a novel dicyanovinyl phenylacetylene, New J. Chem. 41 (2017) 4058-4064, https://doi.org/10.1039/c6nj03794a.
[43] H. Kwon, K. Lee, H.-J. Kim, Coumarin–malonitrile conjugate as a fluorescence turn-on probe for biothiols and its cellular expression, Chem Commun 47 (2011) 1773-1775, https://doi.org/10.1039/c0cc04092d.
[44] J. Mei, J. Z. Sun, A. Qin, B. Z. Tang, Comparative study of the dicyanovinyl-functionalized 1,1-dimethyl-2,3,4,5-tetraphenylsilole derivatives on their structures, properties, and applications in thiol detection, Dyes Pigm. 141 (2017) 366-378, https://doi.org/10.1016/j.dyepig.2017.02.039.
[45] X. Lou, Y. Hong, S. Chen, C. W. T. Leung, N. Zhao, B. Situ, J. W. Y. Lam, B. Z. Tang, A selective glutathione probe based on AIE fluorogen and its application in enzymatic activity assay, Sci. Rep. 4 (2014)

4272-4272, https://doi.org/10.1038/srep04272.
[46] P. V. Bedworth, Y. Cai, A. Jen, S. R. Marder, Synthesis and relative thermal stabilities of diphenylamino- vs piperidinyl-substituted bithiophene chromophores for nonlinear optical materials, J. Org. Chem. 61 (1996) 2242-2246, https://doi.org/10.1021/jo9517555.
[47] F. Steybe, F. Effenberger, S. Beckmann, P. Krämer, C. Glania, R. Wortmann, Enhanced nonlinear optical properties and thermal stability of donor-acceptor substituted oligothiophenes, Chem. Phys. 219 (1997) 317-331, https://doi.org/10.1016/S0301-0104(97)00103-1.
[48] F. Steybe, F. Effenberger, U. Gubler, C. Bosshard, P. Günter, Highly polarizable chromophores for nonlinear optics: syntheses, structures and properties of donor-acceptor substituted thiophenes and oligothiophenes, Tetrahedron 54 (1998) 8469-8480, https://doi.org/10.1016/S0040-4020(98)00450-5.
[49] M. M. M. Raposo, G. Kirsch, Formylation, dicyanovinylation and tricyanovinylation of 5-alkoxy- and 5-amino- substituted 2,2′ -bithiophenes, Tetrahedron 59 (2003) 4891-4899, https://doi.org/10.1016/S0040-4020(03)00705-1.
[50] F. Effenberger, F. Wuerthner, F. Steybe, Synthesis and solvatochromic properties of donor-acceptor-substituted oligothiophenes, J. Org. Chem. 60 (1995) 2082-2091, https://doi.org/10.1021/jo00112a032.
[51] K. Eckert, A. Schröder, H. Hartmann, Preparation and characterization of cyanovinyl-substituted 2-aminothiophenes and 2-aminothiazoles and some of their heterooligomers, Eur. J. Org. Chem. 2000 (2000) 1327-1334, https://doi.org/10.1002/1099-0690(200004)2000:7<1327::aid-ejoc1327>3.0.co;2-t.
[52] M. M. Oliva, J. Casado, M. M. M. Raposo, A. M. C. Fonseca, H. Hartmann, V. Hernández, J. T. López Navarrete, Structure−property relationships in push−pull amino/cyanovinyl end-capped oligothiophenes: Quantum chemical and experimental studies, J. Org. Chem. 71 (2006) 7509-7520, https://doi.org/10.1021/jo060318v.
[53] N. Ikemoto, I. Estevez, K. Nakanishi, N. Berova, Fluorescent bithiophene chromophores: Synthesis and application in CD exciton chirality studies, Heterocyles 46 (1997) 489-501, https://doi.org/10.3987/com-97-S57.
[54] S. Zrig, G. Koeckelberghs, T. Verbiest, B. Andrioletti, E. Rose, A. Persoons, I. Asselberghs, K. Clays, Λ-type regioregular oligothiophenes: Synthesis and second-order NLO properties, J. Org. Chem. 72 (2007) 5855-5858, https://doi.org/10.1021/jo070888a.
[55] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, Williams, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, D. J. Fox. Gaussian 16 Rev. C.01. Wallingford, CT2016.
[56] C. Adamo, V. Barone, Toward reliable density functional methods without adjustable parameters: The PBE0 model, The Journal of Chemical Physics 110 (1999) 6158-6170, https://doi.org/10.1063/1.478522.
[57] V. Barone, M. Cossi, Quantum Calculation of Molecular Energies and Energy Gradients in Solution by a Conductor Solvent Model, The Journal of Physical Chemistry A 102 (1998) 1995-2001, https://doi.org/10.1021/jp9716997.
[58] M. Cossi, N. Rega, G. Scalmani, V. Barone, Energies, structures, and electronic properties of molecules in solution with the C-PCM solvation model, Journal of Computational Chemistry 24 (2003) 669-681,

https://doi.org/https://doi.org/10.1002/jcc.10189.
[59] T. Yanai, D. P. Tew, N. C. Handy, A new hybrid exchange–correlation functional using the Coulomb-attenuating method (CAM-B3LYP), Chemical Physics Letters 393 (2004) 51-57, https://doi.org/https://doi.org/10.1016/j.cplett.2004.06.011.
[60] T. Le Bahers, C. Adamo, I. Ciofini, A Qualitative Index of Spatial Extent in Charge-Transfer Excitations, Journal of Chemical Theory and Computation 7 (2011) 2498-2506, https://doi.org/10.1021/ct200308m.
[61] M. M. M. Raposo, G. Kirsch, A combination of Friedel-Crafts and Lawesson reactions to 5-substituted 2,2'-bithiophenes, Heterocyles 55 (2001) 1487-1498, https://doi.org/10.3987/com-01-9249.