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Vinyl-diazine triphenylamines and their N-methylated derivatives: Synthesis, photophysical properties and application for staining DNA

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ABSTRACT

In this paper we describe the synthesis of vinyl-diazine triphenylamines. These compounds exhibit strong green–yellow fluorescence in dichloromethane solution, important emission solvatochromism and acidochromism. Regioselective N-alkylation of these dyes provides cationic compounds that exhibit affinity for double-stranded DNA. Binding to the biopolymer results in a strong bathochromic shift and increase of the emission intensity.

1. Introduction

Over the past decade, there has been a great interest in the conception of fluorescent probes for the detection of nucleic acids [1]. Among the analytic applications of these probes, fluorescent labeling of DNA is widely used in microscopy to stain the nuclei of cells. The requested characteristics for a good fluorescent DNA probe are a better affinity for DNA than for other cell components, a large increase in quantum yield upon binding with DNA, a low mutagenicity and strong emission inside what is currently known as the physiological optical window between 700 and 1300 nm where biological tissues absorb less [2]. In a previous paper some of us described the synthesis and optical properties of two vinylpyridinium triphenylamines DNA stainers, **TP-2Py** and **TP-3Py** (Fig. 1) [3].

Triphenylamine derivatives are well-known as fluorescent materials. Even if the quantum yield of the triphenylamine itself is not high, judiciously substituted derivatives with strong electron-acceptor groups and π-conjugation extension exhibit strong emission properties with high quantum yields and Stokes shifts [4]. Such structures have found applications as chemosensors [5], components for Organic Light Emitting Diodes (OLEDs) [5d,6], Photovoltaic materials [7] and Two-Photon Absorption (TPA) chromophores [4ab,8].

Recently, diazine rings and in particular pyrimidine and pyrazine heterocycles, have been subject to intensive research, due to their interest as building blocks for the synthesis of functionalized π-conjugated materials [9]. In particular, star-shaped and V-shaped structures with a pyrimidine central core substituted with electron-withdrawing groups exhibit intense fluorescence emission [10] and TPA properties [11]. Linear [12] as well as V-shaped [13] structures incorporating a pyrazine moiety exhibit also interesting emission properties. Therefore, we speculated that the combination of the two scaffolds will afford compounds of practical interest for fluorescence applications.

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The aim of this paper is to describe the synthesis and photophysical properties of new chromophores with a triphenylamine core substituted with vinyldiazines and diazinium moieties at the periphery. Application of the vinylpyrimidinium triphenylamine derivatives to DNA labeling is also discussed.

2. Experimental

2.1. General experimental

All solvents (reagent grade) and the starting materials were acquired from Sigma–Aldrich and were used without purification. Column chromatography was performed with the indicated solvents using Acros neutral aluminum oxide Brockmann I (particle size 50–200 μm). Yields refer to chromatographically and spectroscopically pure compounds. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.3 MHz respectively, on a Bruker AC-300 spectrometer at room temperature using an internal deuterium lock. Chemical shift values are given in ppm relative to tetramethyl silane (TMS). Acidic impurities in $CDCl_3$ were removed by treatment with anhydrous K_2CO_3. Assignments of the resonance to individual protons were based on integration and selective homonuclear correlation (COSY). Heteronuclear multiple coherence (HSQC, HMBC) spectra were obtained for all compounds and the carbon resonances were assigned. Melting points were determined on Electrothermal 1100 element. Quantitative UV–visible spectra were recorded with a UVIKON xm SECOMAM spectrometer. Fluorescence spectra were recorded using Spex FluoroMax-3 Jobin-Yvon Horiba apparatus. Measurements were performed at room temperature with solutions of OD < 0.1 to avoid re-absorption of the emitted light, and data were corrected with a blank and from the variations of the detector with the emitted wavelength. Fluorescence quantum yield were measured according to Crosby comparative method [14] using quinine bisulfate in 1 M H_2SO_4 ($\Phi_F = 0.54$) or rhodamine in ethanol ($\Phi_F = 1.00$) as reference. The CD spectra were recorded on a Jasco J-710 spectrometer in a 1 cm quartz cuvette thermostated at 20 °C. Microanalyses were obtained from ICSN-CNRS Elemental Analysis Center at Gif-sur-Yvette, France. Mass spectrometry (Electrospray Ionization, ESI) was performed using ZQ 2000 Waters apparatus.

2.2. Studies of interactions with DNA

The fluorimetric titrations were performed at a constant dye concentration (1 μM) with increasing concentrations of DNA and the binding curves were obtained by plotting the fluorescence enhancement F/F_0 (F = integrated fluorescence area of the complex and F_0 = integrated fluorescence area of the free dye) versus the concentration in DNA. The DNA used is drewAT, which is a 14 base pairs oligonucleotide CGCGAAATTTCGCG.

2.3. General procedure for the dicondensation of aldehydes **1** and **2** and methyldiazine

A stirred mixture of methyldiazine (2.2 mmol) and the corresponding 4,4′-diformyltriphenylamine (1.0 mmol) in aqueous sodium hydroxide (5 M, 15 mL) containing aliquat 336 (43 mg, 0.1 mmol) was heated under reflux for 1 h (4 h for compound **7**) The mixture was allowed to cool and the precipitate was filtered off, washed with water, and purified either by crystallization from the indicated solvent or chromatography on alumina gel column.

2.3.1. Synthesis of 4,4′-di[(E)(pyrimidin-4-yl)vinyl]triphenylamine (**3**)

Condensation reaction according to general procedure gave after purification by crystallization from MeOH/water, 321 mg (71%) of **3** as a yellow solid. Mp: 207–208 °C. ^{1}H NMR ($CDCl_3$): 9.14 (s, 2H, H-2_{pyrim}), 8.64 (d, 2H, J = 5.1 Hz, H-5_{pyrim}), 7.84 (d, 2H, J = 15.9 Hz, H-$_{vinylpyrim}$), 7.50 (d, 4H, J = 8.4 Hz, H-m_{NPh3}), 7.33–7.27 (m, 3H, H-5_{pyrim} + H-m_{NPh3}), 7.18–7.10 (m, 7H, H-o_{NPh3} + H-p_{NPh3}), 6.95 (d, 2H, J = 15.9 Hz, H-$_{vinylNPh3}$) ^{13}C ($CDCl_3$): 162.4 (C-4_{pyrim}), 158.9(C-2_{pyrim}), 157.3(C-6_{pyrim}), 148.4 (C-1_{NPh3}), 146.5 (C-1_{NPh3}), 136.8 (C-vinyl$_{pyrim}$), 130.0 (C-4_{NPh3}), 129.7(C-m_{NPh3}), 128.9 (C-m_{NPh3}), 125.8 (C-p_{NPh3}), 124.6 (C-vinyl$_{pyrim}$), 123.8 (C-o_{NPh3}), 123.5 (C-o_{NPh3}), 118.5(C-5_{pyrim}). MS (ES^+): 453.1 (M^+, 100%). Anal. Cald for $C_{30}H_{23}N_5$ (453.54): C, 79.45; H, 5.11; N, 15.44; Found C, 79.23; H, 5.53; N, 15.31.

2.3.2. Synthesis of 4,4′-di[(E)(2-thiomethylpyrimidin-4-yl)vinyl] triphenylamine (**4**)

Condensation reaction according to general procedure gave after purification by column chromatography (ethyl acetate: *n*-hexane 2:3), 109 mg (20%) of **4** as a yellow solid. Mp: 149–151 °C. ^{1}H NMR ($CDCl_3$) δ 8.43 (d, 2H, J = 5.2 Hz, H-5_{pyrim}), 7.82 (d, 2H, J = 15.7 Hz, H-$_{vinylpyrim}$), 7.48 (d, 4H, J = 8.7 Hz, H-m_{NPh3}), 7.16–7.10 (m, 7H, J = 8.6 Hz, H-o_{NPh3} + H-p_{NPh3}), 6.93 (d, 2H, J = 5.2 Hz, H-6_{pyrim}), 6.88 (d, J = 15.8 Hz, H-vinyl$_{NPh3}$), 2.62 (s, 6H, Me). ^{13}C NMR ($CDCl_3$) δ 172.33 (C-3_{pyrim}), 162.55 (C-1_{pyrim}), 157.28 (C-5_{pyrim}), 148.31 (C-1_{NPh3}), 146.53 (C-1_{NPh3}), 136.82 (C-vinyl$_{pyrim}$), 130.05 (C-4_{NPh3}), 129.64 (C-m_{NPh3}), 128.84 (C-m_{NPh3}), 125.76 (C-p_{NPh3}), 124.50 (C-vinyl$_{pyrim}$), 123.79 (C-o_{NPh3}), 123.50 (C-o_{NPh3}), 113.64 (C-6_{pyrim}), 14.16 (C-Me). MS (ES^+): 546 (M^+). HRMS (ESI) Anal. Cald for $C_{32}H_{27}N_5S_2$ $[M + H]^+$ 546.1781, found 546.1788.

2.3.3. Synthesis of 4-bromo-4′,4″-di[(E)(pyrimidin-4-yl)vinyl] triphenylamine (**5**)

Condensation reaction according to general procedure gave after purification by column chromatography (ethyl acetate: *n*-heptane 2:3), 225 mg (42%) of **5** as a yellow solid. Mp: 237–238 °C. ^{1}H NMR ($CDCl_3$): 9.15 (s, 2H, H-2_{pyrim}), 8.65 (d, 2H, J = 5.1 Hz, H-6_{pyrim}), 7.84 (d, 2H, J = 15.9 Hz, H-$_{vinylpyrim}$), 7.51 (d, 4H, J = 8.4 Hz, H-m_{NPh3}), 7.42 (d, 2H, J = 8.4 Hz, H-m_{NPh3}), 7.29 (d, 2H, J = 5.1 Hz, H-5_{pyrim}), 7.09 (d, 4H, J = 8.4 Hz, H-o_{NPh3}), 7.04 (d, 2H, J = 8.4 Hz, H-o_{NPh3}), 6.95 (d, 2H, J = 15.9 Hz, H-$_{vinylNPh3}$) ^{13}C ($CDCl_3$): 162.3 (C-4_{pyrim}), 158.9(C-2_{pyrim}), 157.3(C-6_{pyrim}), 147.9 (C-1_{NPh3}), 145.7 (C-1_{NPh3}), 136.6 (C-vinyl$_{pyrim}$), 132.7(C-m_{NPh3}), 130.5 (C-4_{NPh3}), 128.9 (C-m_{NPh3}), 126.8 (C-vinyl$_{pyrim}$), 124.1 (C-o_{NPh3}), 123.8 (C-o_{NPh3}), 118.6(C-5_{pyrim}), 117.0 (C–Br). MS (ES^+): 532.3 (MH^+, 100%), 534.3

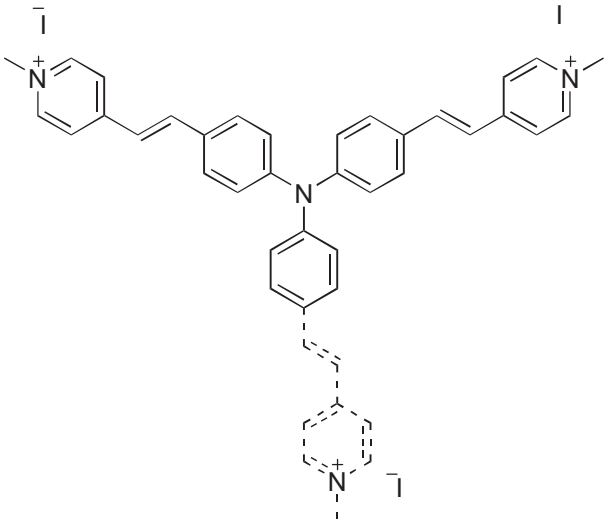


Fig. 1. Structure of model compounds **TP-2Py** (solid) and **TP-3Py** (dashed) previously developed as DNA stainers.

(MH^+, 100%). Anal. Cald for $C_{30}H_{22}BrN_5$ (532.43): C, 67.67; H, 4.16; N, 13.15; Found C, 67.29; H, 4.43; N, 13.31.

*2.3.4. Synthesis of 4,4′-di[(E)(pyrazin-2-yl)vinyl]triphenylamine (**6**)*

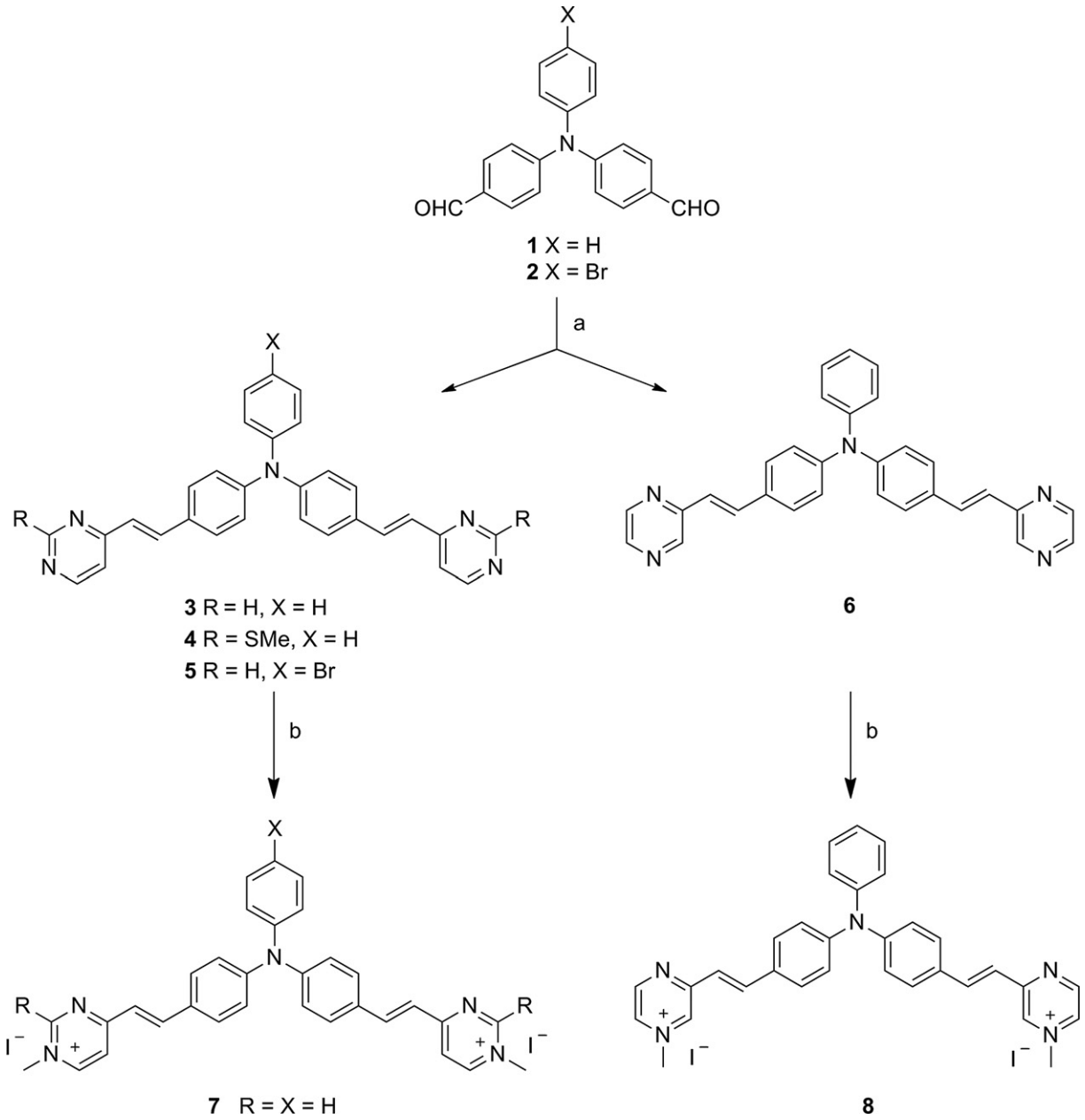
Condensation reaction according to general procedure gave after purification by column chromatography (ethyl acetate: *n*-heptane 2:3), 228 mg (51%) of **6** as a yellow solid. Mp: 152–153 °C. ^{1}H NMR ($CDCl_3$): 8.61 (s, 2H, H-3_{pyra}), 8.52 (s, 2H, H-6_{pyra}), 8.38 (s, 2H, H-5_{pyra}), 7.70 (d, 2H, J = 15.9 Hz, H-$_{vinylpyrim}$), 7.49 (d, 4H, J = 8.4 Hz, H-m_{NPh3}), 7.34–7.29 (m, 2H, H-m_{NPh3}), 7.18–7.09 (m, 7H, H-o_{NPh3} + H-p_{NPh3}), 7.05 (d, 2H, J = 15.9 Hz, H-$_{vinylNPh3}$) ^{13}C ($CDCl_3$): 151.6 (C-2_{pyra}), 148.0(C-1_{NPh3}), 146.8 (C-1_{NPh3}), 144.3 (C-6_{pyra}), 143.6 (C-3_{pyra}), 142.4 (C-2_{pyra}), 134.5 (C-vinyl$_{NPH3}$), 130.5 (C-4_{NPh3}), 129.6 (C-m_{NPh3}), 128.4 (C-m_{NPh3}), 125.6 (C-p_{NPh3}), 124.3 (C-o_{NPh3}), 123.7 (C-p_{NPh3}), 122.4 (C-vinyl$_{pyra}$). MS (ES^+): 453.1 (100%, M^+), 454.2 (55%, MH^+) Anal. Cald for $C_{30}H_{23}N_5$ (453.54): C, 79.45; H, 5.11; N, 15.44; Found C, 79.40; H, 5.15; N, 15.44.

*2.3.5. Synthesis of 4,4′-di[(E)(1methylpyrimidin-4-ium)vinyl] triphenylamine (**7**)*

A mixture of compound **3** (20 mg, 0.04 mmol) and methyliodide (1 mL) was stirred for 3 h. Compound **7** was filtrated and obtained as a red solid. ^{1}H NMR (DMSO): δ 9.48 (s, 2H), 9.04 (d, J = 5.8 Hz, 2H), 8.28 (d, J = 15.9 Hz, 2H), 8.12 (d, J = 6.5 Hz, 2H), 7.83 (d, J = 8.0 Hz, 4H), 7.51–7.41 (m, 4H), 7.31 (t, J = 7.7 Hz, 2H), 7.21 (d, J = 7.2 Hz, 2H), 7.12 (d, J = 8.0 Hz, 4H), 4.14 (s, 6H). HRMS (ESI) Anal. Cald for $C_{32}H_{29}N_5$ [M^+] 483.2417, found 483.2423.

*2.3.6. Synthesis of 4,4′-di[(E)(1methylpyrazin-4-ium)vinyl] triphenylamine (**8**)*

A mixture of compound **6** (20 mg, 0.04 mmol) and methyliodide (1 mL) was stirred for 3 h. Compound **8** was filtered and obtained as a red solid. ^{1}H NMR (300 MHz, DMSO) δ 9.35 (s, 1H), 9.27 (s, 1H), 8.86 (s, 1H), 7.98 (d, J = 15.7 Hz, 2H), 7.73 (d, J = 7.5 Hz, 4H), 7.24 (t, J = 7.0 Hz, 1H), 7.19 (d, J = 7.5 Hz, 2H), 7.11 (d, J = 7.5 Hz, 4H), 4.36 (s,



Scheme 1. Synthesis of the triphenylamine based chromophores. a) aqueous NaOH, aliquat 336, reflux 1 h; b) CH_3I 3 h.

6H). HRMS (ESI) Anal. Cald for $C_{32}H_{29}N_5$ [M^+] 483.2417, found 483.2423.

3. Results and discussion

3.1. Synthesis

The main method described in the literature for the synthesis of (E)-vinyldiazines consists in the condensation of aldehydes with the corresponding methyldiazines [15,13c,10d]. This approach has the advantages of a large range of commercially available or easily accessible methyldiazines and the use of environmentally friendly conditions. Triphenylamine building blocks **2** and **1** have been obtained in good yield from triphenylamine according to procedures described in the literature [16]. Condensation reactions have been carried out with various pyrimidine derivatives and 2-methylpyrazine on compounds **1** and **2** in boiling aqueous 5 M NaOH using aliquat 336 as catalyst, according to a previously reported procedure [15a]. Compounds **3**–**6** have been obtained in good yields (Scheme 1). The N-methylation has been carried out with iodomethane at reflux without solvent or under microwave activation. As described in the literature [17]. N-methylation occurs regioselectively on the diazine rings and dimethylated products **7**–**8** are obtained in good yield.

3.2. UV/vis and PL spectroscopy

The optical properties of the synthetized vinyl-diazine triphenylamine derivatives **3**–**6** have been investigated by UV/visible and photoluminescence spectroscopy on CH_2Cl_2 solutions at room temperature. The data obtained are summarized in Table 1. All compounds were photostable and did not undergo cis–trans isomerization under the analysis conditions. All the compounds show similar absorption spectra with two bands: one in the visible region of the spectrum (417–422 nm) and a second one of higher energy (286–292 nm). Neutral derivatives show high fluorescence emission in CH_2Cl_2, with quantum yields ranging from 0.60 to 0.94. In particular, the emission band of the pyrazine derivative **6** exhibits a large bathochromic shift and high fluorescence quantum yield compared with pyrimidine derivatives **3**–**5**. As an example, the spectra of compound **3** are shown in Fig. 2 (blue line). Compounds **3**, **4** and **5** show similar emission properties with quantum yields between 0.6 and 0.7. Large Stokes shifts are observed for the compounds under investigation, indicating important differences (vibrational, electronic, geometric) between the Franck–Condon state and the excited state from which the emission starts.

A fluorosolvatochromic study has been carried out on compound **3** (Fig. 3). A bathochromic shift of the emission band can be observed while increasing solvent polarity, estimated by solvent

Table 1
UV/Vis and photoluminescence data of neutral compounds **3**–**6** and **TP-2Py**.

Compound[a]	λ_{abs}, nm (ε, L mol^{-1} cm^{-1})		λ_{em}, nm	Φ_F[b]	Φ_Δ[c]	SS[d], cm^{-1}
3	288 (52,300)	422 (29,500)	555	0.73	0.07	5679
4	286 (26,625)	430 (42,420)	540	0.60	0.07	4737
5	292 (46,700)	417 (27,900)	555	0.69	–	5963
6	292 (57,900)	420 (35,800)	569	0.94	0.08	6235
TP-2Py	–	402 (50,350)	502	0.64	0.03	4955

[a] All spectra were recorded in CH_2Cl_2 solutions at room temperature from $c = 5.0 \times 10^{-6}$ M to 1.0×10^{-5} M.
[b] Fluorescence quantum yield (±10%); excitation at 420 nm.
[c] Singlet oxygen quantum yields were measured relative to rose bengale in CH_2Cl_2 as standard ($\Phi_\Delta = 0.76$); excitation at 420 nm.
[d] Stokes shift.

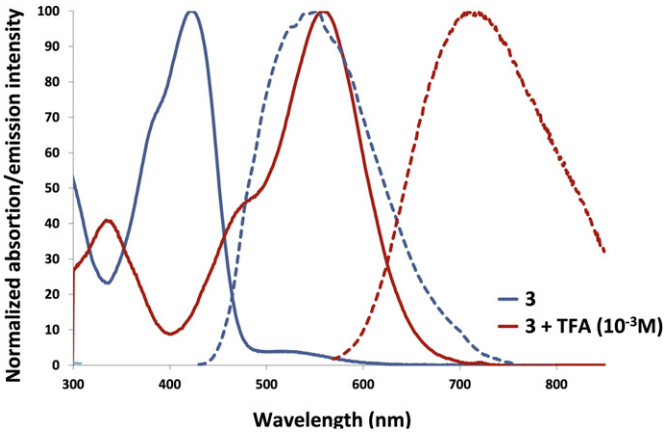


Fig. 2. Normalized absorption (solid line) and emission (broken line) of compound **3** in CH_2Cl_2 with (red) or without (blue) addition of TFA. $c = 5.0 \times 10^{-6}$ M. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

orientation polarizability [18.] In contrast, the absorption band is not significantly shifted. This solvatochromic behavior is typical of compounds that undergo an internal charge transfer upon excitation leading to a highly polar charge separated emitting state stabilized by polar solvents. This phenomenon has been fully documented with donor–acceptor fluorophores [19,4b,10d].

As well, the nitrogen atoms of the prepared diazines are basic centers that can be protonated (pKa = 1.2 in water) [10d,13c,15d]. Thus, the effect of protonation on the optical properties of compounds **3** and **6** in CH_2Cl_2 has been studied. The changes in the UV–vis spectra of **3** upon addition of trifluoroacetic acid are illustrated in Fig. 2. The spectra show the disappearance of the absorption band at 400 nm upon addition of acid, whereas a new band corresponding to the protonated species appears at 570 nm. Similar changes have been observed for compound **6**. Concerning the photoluminescence, it is interesting to note that the protonated form of pyrimidine derivative **3** remains emissive with a red-shifted fluorescence, as it is illustrated in broken line in Fig. 2, while pyrazine derivative **6** becomes nonemissive.

The optical properties of pyrimidinium and pyrazinium salts **7** and **8** have been investigated in different solvents: water, glycerol and DMSO (Table 2). As observed for model compound **TP-2Py**, the new compounds are not fluorescent in aqueous media. This is due to the existence of nonradioactive decay processes presumably

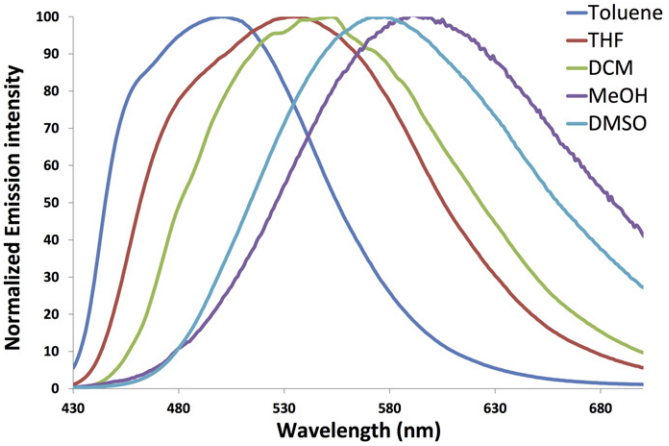


Fig. 3. Normalized emission of compound **3** in various solvents. $c = 5.0 \times 10^{-6}$ M.

Table 2
UV/Vis and photoluminescence data of salts **7**, **8** and **TP-2Py**.

Compound	Solvent[a]	λ_{abs}, nm (ε, L mol^{-1} cm^{-1})	λ_{em}, nm	Φ_F[b]	SS[c], cm^{-1}
7	Buffer	525 (59,810)	–	–	–
	Glycerol	560 (76,640)	685	0.026	3259
	DMSO	500 (45,601)	720	<0.01	6519
8	Buffer	485 (33,980)	–	–	–
	Glycerol	500 (38,400)	690	<0.01[d]	5507
	DMSO	440 (57,340)	530	<0.01	3859
TP-2Py	Buffer	509 (31,400)	656	0.08	4402
	Glycerol	496 (37,400)	649	0.11	4753

[a] All spectra were recorded at room temperature and the concentration range was $c = 3 \times 10^{-6}$ M–15×10^{-5} M in lithium cacodylate buffer pH 7.2, 100 mM NaCl, $c = 0.5 \times 10^{-6}$ M–1.5×10^{-5} M in glycerol and $c = 0.5 \times 10^{-6}$ M–3×10^{-5} M in DMSO.
[b] Fluorescence quantum yield (±10%).
[c] Stokes shift.
[d] Quantum yield obtained 0.001.

involving molecular motions around the vinyl bond and also to interactions with water. The optical properties of these salts have also been studied in glycerol, which is a viscous solvent that hinders the vibration of the molecule. A weak fluorescence is observed in these conditions, with quantum yields of 2.6% and 0.1% for compounds **7** and **8** respectively. If we compare the maximum absorption wavelength in glycerol of both pyrimidine and pyrazine derivatives with that of the **TP-2Py** we observe that there is a bathochromic shift, specially important in the case of the pyrimidime derivative **7** (60 nm). As well, the molar absorption coefficients (ε) of the diazine derivatives are higher than that of the **TP-2Py**, being that more than twice in the case of compound **7** (76,640 M^{-1} cm^{-1} for **7** versus 37,400 M^{-1} cm^{-1} for **TP-2Py**). Concerning the emission properties in glycerol, both **7** and **8** emit at longer wavelengths than the **TP-2Py**, with respective shifts of 36 and 41 nm. However, the values of the quantum yields for the diazine derivatives are very low compared to that of the **TP-2Py**. The optical properties of these compounds have also been studied in DMSO. In this case, we can observe that the maximum absorption wavelength is lower than in glycerol or aqueous media for both **7** and **8**, with very low quantum yields. In the case of pyrimidine salt **7**, the maximum emission wavelength is strongly shifted to the red compared with that in glycerol. Moreover, the Stokes shift of **7** in DMSO is especially high (6519 cm^{-1}).

The production of singlet oxygen by pyrimidine derivatives **3** and **4** as well as by pyrazine derivative **6** have been studied and quantum yields between 0.07 and 0.08 were found (Table 1), which is of the same order as for the previously reported molecule **TP-2Py** (0.03). The singlet oxygen quantum yields obtained for compounds **3**, **4** and **6** are too weak to consider these molecules as potential photosensitizers. Nevertheless, it is worth noting that no significant heavy atom effect was observed for **4** despite the presence of the sulfur atoms on the pyrimidine rings.

3.3. Interaction with DNA

As it was explained before [3], **TP-2Py** is virtually non fluorescent when free in water, whilst a strong fluorescence enhancement is observed upon binding to DNA making this molecule

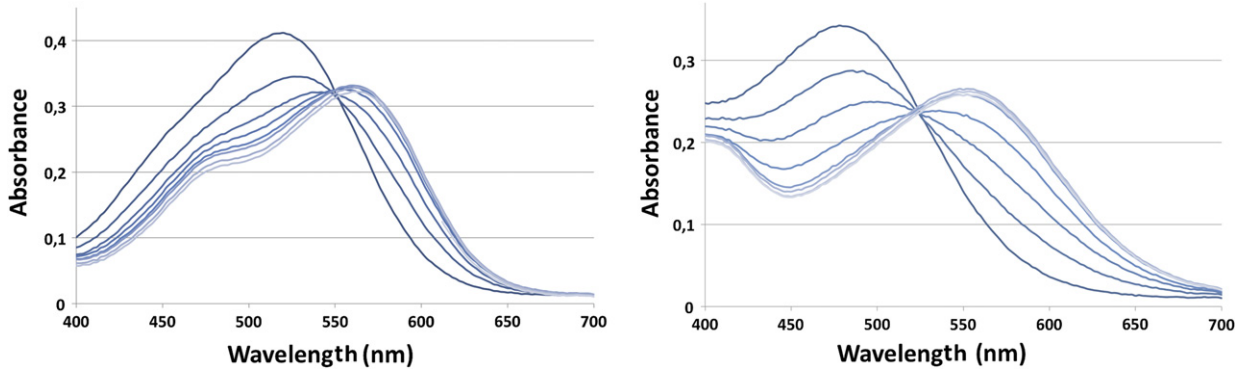


Fig. 4. Absorbance of compound **7** (left) and **8** (right) in sodium cacodylate buffer pH 7.2, 100 mM NaCl upon addition of 0–5 molar equivalents of drewAT $c = 1.0 \times 10^{-5}$ M.

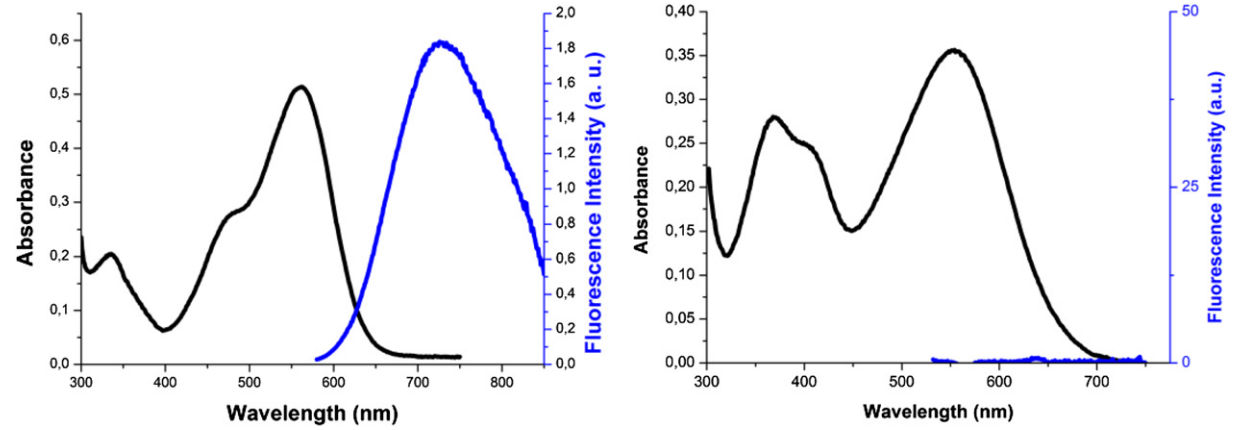


Fig. 5. Absorption (black) and emission (blue) spectra of compound **7** (left) and **8** (right) in sodium cacodylate buffer pH 7.2, 100 mM NaCl in presence of 1 molar equivalent of drewAT. $c_{abs} = 10$ μM and $c_{em} = 1$ μM. Excitation at 560 nm for compound **7** and at 510 for compound **8**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Optical properties of the cationic dyes in the presence of 5 molar equivalents of drewAT.

Compound[a]	λ_{abs}, nm (ε, L mol^{-1} cm^{-1})	λ_{em}, nm	Φ_F[b]	ΔS[c], cm^{-1}
7	560 (38,330)	725	<0.01[d]	4064
8	550 (28,870)	–	–	–
TP-2Py	509 (31,400)	656	0.08	4403

[a] All spectra were recorded in lithium cacodylate buffer pH 7.2, 100 mM NaCl at room temperature at $c = 0.5 \times 10^{-6}$ M–2.5×10^{-5} M.
[b] Fluorescence quantum yield (±10%).
[c] Stokes shift.
[d] Exact value of the quantum yield of **7** in these conditions was 0.004.

a remarkable DNA light-up probe. The fluorescence restoration of **TP-2Py** bound to DNA, is attributed to the restriction of molecular motions around the vinyl bond and to hydrophobic effects namely screening from water once the dye is inserted inside DNA. This emission specifically turned-on in presence of the biopolymeric matrix is a clear advantage for cellular imaging of nuclear DNA or chromosomes, as it should result in a high imaging contrast.

It was therefore interesting to evaluate the affinity of the analogs **7** and **8** for DNA. In this aim, the UV–vis titration of both salts **7** and **8** was carried out. Fig. 4 depicts the absorbance of compounds **7** and **8** upon addition of DNA, pointing out a bathochromism in absorbance typical of DNA interaction. A clear isobestic point is obtained at 522 nm for compound **8** and at 552 nm for compound **7** reflecting the existence of two forms in equilibrium.

For compound **7**, a fluorescence signal is observed upon addition of one equivalent of DNA (Fig. 5). Likely, the DNA matrix plays the same role as glycerol by hindering the vibration mode around double bonds thereby increasing the fluorescence. In these conditions, the Stokes shift is important (3300 cm^{-1}) and the emission wavelength is red-shifted as compared to the **TP-2Py** analog (725 nm versus 650 nm). The quantum yield obtained for the pyrimidinium salt **7** in presence of an excess of DNA (Table 3) albeit moderate is significant (0.4%). In contrast, no fluorescence restoration is observed for the pyrazinium salt **8** upon addition of DNA (Fig. 5).

This result is not unexpected as compound **8** was found to be poorly fluorescent in glycerol ($\Phi_F = 0.001$ versus $\Phi_F = 0.026$ for compound **7**, Table 2). Nonetheless, an interaction between **8** and DNA occurs as indicated by the red-shift and the pronounced hypochromism of absorbance in presence of DNA (Fig. 4).

The fluorimetric titration of salt **7** with drewAT (Fig. 6) shows a quite important fluorescence enhancement (F/F_0), around 35. This

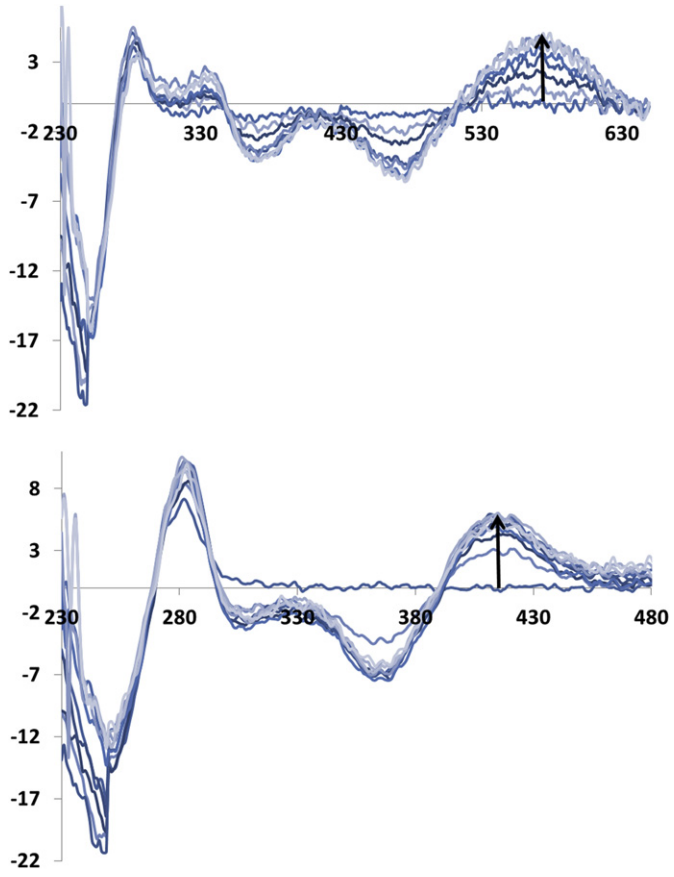


Fig. 7. Circular dichroism spectra of drewAT [at 3 mM] upon addition of compound **7** (up) **8** (down); concentration in dye (0–16 mM) in lithium cacodylate buffer, 10 mM, pH 7.2, 100 mM NaCl.

fluorimetric titration has been fitted with a one-site model in order to determine the affinity constant ($K_a = 8.9 \times 10^5$ M^{-1}).

As well, Circular Dichroism (CD) measurements were performed with salts **7** and **8** in presence of drewAT at increasing dye/DNA ratios. In these conditions, a strong induced CD (ICD) signal is observed for both compounds. This signature that reflects the association to the chiral DNA matrix, is more frequently observed with dyes binding in the minor groove of the double helix [3,20] (Fig. 7).

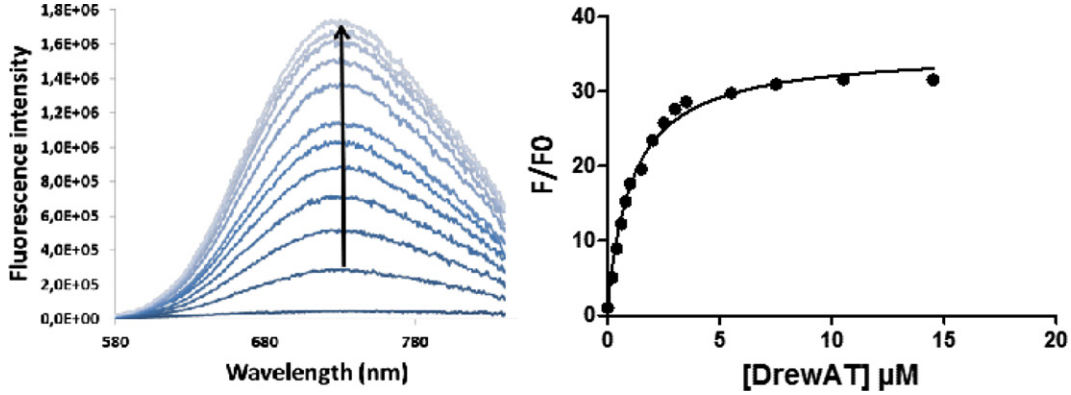


Fig. 6. (left) Fluorimetric titration of drewAT with compound **7** $c = 2$ µM in 10 mM lithium cacodylate buffer pH 7.2, 100 mM NaCl, [drewAT] = 0–15 µM and (right) fluorescence enhancement fitting of compound **7** upon addition of drewAT.

4. Conclusion

Four divinyldiazine-triphenylamine **3**–**6** derivatives have been obtained in good yield from 4,4′-diformyltriphenylamine derivatives. Both pyrimidine and pyrazine derivatives exhibit strong fluorescence in dichloromethane with quantum yields up to 0.94. A strong emission solvatochromism and a dramatic color change in acidic media have been observed. This behavior indicates that this type of material could be developed as luminescent polarity and pH sensors. The corresponding N-methyl pyrimidinium and pyrazinium salts (**7** and **8**) have also been synthesized. The two compounds display affinity for double-stranded DNA as demonstrated by UV–vis and CD measurements. The two compounds are not fluorescent in aqueous media but the fluorescence of the pyrimidinium salt **7** is restored when bound to DNA whilst the pyrazinium **8** remains quenched. Remarkably, the emission of **7** associated to DNA is strongly red-shifted peaking in the (very) near infrared region λ_{em} = 725 nm. Altogether, this makes this pyrimidinium derivative a new NIR DNA stainer of potential interest for imaging.

References

[1] (a) Kricka LJ. Stains, labels and detection strategies for nucleic acids assays. Ann Clin Biochem 2002;39:114–29;
(b) Gingeras TR, Higuchi R, Kricka LJ, Lo YMD, Wittwer CT. Fifty years of moleculars (DNA/RNA) diagnostics. Clin Chem 2005;51:661–71.

[2] (a) Waynant RW, Ediger MN, editors. Electrooptics handbook. New York: McGraw-Hill; 1993 [Chapter 24];
(b) Tromberg BJ, Shah N, Lanning R, Cerussi A, Espinoza J, Pham T, et al. Non-Invasive in vivo characterization of breast tumors using photon migration spectroscopy. Neoplasia 2000;2:26–40.

[3] Allain C, Schmidt F, Lartia R, Bordeau G, Fiorini-Debuisschert C, Charra F, et al. Vinyl-pyridinium triphenylamines: novel far-red emitters with high photostability and two-photon absorption properties for staining DNA. Chem Bio Chem 2007;8:424–33.

[4] (a) Porres L, Mongin O, Katan C, Charlot M, Pons T, Mertz J, et al. Enhanced two-photon absorption with novel octupolar propeller-shaped fluorophores derived from triphenylamine. Org Lett 2004;6:47–50;
(b) Lartia R, Allain C, Bordeau G, Schmidt F, Fiorini-Debuisschert C, Charra F, et al. Synthetic strategies to derivatizable triphenylamines displaying high two-photon absorption. J Org Chem 2008;73:1732–44;
(c) Bordeau G, Lartia R, Teulade-Fichou MP. Meta substituted triphenylamines as new dyes displaying exceptionally large stokes shifts. Tetrahedron Lett 2010;51:4429–32.

[5] (a) Gu D, Yang G, He Y, Qi B, Wang G, Su Z. Triphenylamine-based pH chemosensor: synthesis, crystal structure, photophysical properties and computational studies. Synth Met 2009;159:2497–501;
(b) Chi LN, Wu YB, Zhang X, Ji SM, Shao JY, Guo HM, et al. Ethynylated triphenylamine monoboronic acid chemosensors: experimental and theoretical studies. J Fluoresc 2010;20:1255–65;
(c) Feng JC, Li Y, Yang MJ. Hyperbranched copolymer containing triphenylamine and divinyl bipyridyl units for fluorescent chemosensors. J Polym Sci Part A Polym Chem 2009;47:222–30;
(d) Ning Z, Chen Z, Zhang Q, Yan Y, Qian S, Cao Y, et al. Aggregation-induced emission (AIE)-active starburst triarylamine fluorophores as potential non-doped red emitters for organic light-emitting diodes and Cl_2 gas chemodosimeter. Adv Funct Mater 2007;17:3799–807.

[6] (a) Lee KH, You JN, Kwon HJ, Kim YK, Yoon SS. Synthesis and electroluminescent properties of blue fluorescent triphenylamine substituted anthracene derivatives for OLEDs. Mol Cryst Liq Cryst 2010;530:204–11;
(b) Gong SL, Zhao YBA, Wang M, Yang CL, Zhong C, Qin JG, et al. Versatile benzimidazole/triphenylamine hybrids: efficient non doped deep-blue electroluminescence and good host materials for phosphorescent emitters. Chem Asian J 2010;5:2093–9;
(c) Tao S, Zhou YC, Lee CS, Lee ST, Huang D, Zhang XH. Highly efficient non doped blue organic light-emitting diodes based on anthracene–triphenylamine derivatives. J Phys Chem C 2008;112:14603–6.

[7] Ning Z, Tian H. Triarylamine: a promissing core unit for efficient photovoltailc materials. Chem Commun; 2009:5483–95.

[8] (a) Parent M, Mongin O, Kamada K, Katan C, Blanchard-Desce M. New chromophores from click chemistry for two-photon absorption and tunable photoluminescence. Chem Commun; 2005:2029–31;
(b) Katan C, Tretiak S, Werts MHV, Bain AJ, Marsh RJ, Leonczek N, et al. Two-photon transitions in quadrupolar and branched chromophores: experiment and theory. J Phys Chem B 2007;111:9468–83;
(c) Lee HJ, Sohn J, Hwang J, Park SY. Triphenylamine-cored bifunctional organic molecules for two-photon absorption and photoreaction. Chem Mater 2004;16:456–65;
(d) Bordeau G, Lartia R, Metge G, Fiorini-Debuisschert C, Charra F, Teulade-Fichou MP. Trinaphthylamines as robust organic materials for two-photon-induced fluorescence. J Am Chem Soc 2008;130:16836–7;
(e) Jiang Y, Wang Y, Hua J, Tang J, Li B, Qian S, et al. Multibrnached triarylamine end-caped triazines with aggregation-induced emission and large two-photon absorption cross-sections. Chem Commun 2010;46:4689–91;
(f) Wang B, Wang Y, Hua J, Jiang Y, Huang J, Qian S, et al. Starburst triarylamine donor–acceptor–donor quadrupolar derivatives based on cyano-substituted diphenylaminestyrylbenzene: tunable aggregation-induced emission colors and large two-photon absorption cross sections. Chem Eur J 2011;17:2647–55;
(g) Yan Z-Q, Xu B, Dong Y-J, Tian W-J, Li A-W. Dyes Pigm 2011;90:269–74;
(h) Tian YP, Li L, Zhang JZ, Yang JX, Zhou HP, Wu JY, et al. Investigations and facile synthesis of a series of novel multifunctional two-photon absorption materials. J Mater Chem 2007;17:3646–54.

[9] (a) Achelle S, Plé N. Pyrimidine ring as building block for the synthesis of functionalized π-conjugated materials. Curr Org Synth 2012;9:163–87;
(b) Pieterse K, Lauritsen A, Schenning APHJ, Vekemans JAJM, Meijer EW. Symmetrical electron-deficient materials incorporating azaheterocycles. Chem Eur J 2003;9:5597–604.

[10] (a) Itami K, Yamazaki D, Yoshida Ji. Pyrimidine-core extended π-systems: general synthesis and interesting fluorescent properties. J Am Chem Soc 2004;126:15396–7;
(b) Achelle S, Ramondenc Y, Marsais F, Plé N. Star- and banana-shaped oligomers with a pyrimidine core: synthesis and light-emitting properties. Eur J Org Chem; 2008:3129–40;
(c) Achelle S, Ramondenc Y, Dupas G, Plé N. Bis- and tris(arylethynyl)pyrimidine oligomers: synthesis and light-emitting properties. Tetrahedron 2008;64:2783–91;
(d) Achelle S, Nouira I, Pfaffinger B, Ramondenc Y, Plé N, Rodríguez-López J. V-shaped 4,6-bis(arylvinyl)pyrimidine oligomers: synthesis and optical properties. J Org Chem 2009;74:3711–7.

[11] (a) Liu Z, Shao P, Huang Z, Liu B, Chen T, Qin J. Two-photon absorption enhancement induced by aggregation due to intermolecular hydrogen bonding in V-shaped 2-hydroxypyrimidine derivatives. Chem Commun; 2008:2260–2;
(b) Li L, Tian YP, Yang JX, Sun PP, Wu JY, Zhou HP, et al. Facile synthesis and systematic investigations of a series of novel bent-shaped two-photon absorption chromophores based on pyrimidine. Chem Asian J 2009;4:668–80;
(c) Akdas-Kilig H, Roisnel T, Ledoux I, Le Bozec H. A new class of bipyrimidine-based octupolar chromophores: synthesis, fluorescent and quadratic nonlinear optical properties. New J Chem 2009;33:1470–3;
(d) Achelle S, Saettel N, Baldeck P, Teulade-Fichou MP, Maillard P. Bisporphyrin connected by pyrimidine: synthesis and photophysical properties. J Porphyrins Phthalocyanines 2010;14:877–84.

[12] (a) Türksoy F, Hughes G, Batsanov AS, Bryce MR. Phenylene-2,5-dimethylpyrazine co-oligomers: synthesis by suzuki couplings, X-ray structures of neutral and diprotonated species and efficient blue emission. J Mater Chem 2003;13:1554–7;
(b) Hebbar N, Ramondenc Y, Plé G, Dupas G, Plé N. Push–pull structures with a pyrazine core and hexatriene chain: synthesis and light-emitting properties. Tetrahedron 2009;65:4190–200;
(c) Saito R, Matsumura Y, Suzuki S, Okazaki N. Intensely blue-fluorescent 2,5-bis(benzoimidazol-2-yl)pyrazine dyes with improved solubility: their synthesis, fluorescent properties, and application as microenvironment polarity probes. Tetrahedron 2010;66:8273–9;
(d) Zhao L, Perepichka IF, Türksoy F, Batsanov AS, Beeby A, Findlay KS, et al. 2,5-Di(aryleneethynyl)pyrazine derivatives: synthesis, structural and opto-electronic properties and light-emitting device. New J Chem 2004;28:912–8;
(e) Hebbar N, Fiol-Petit C, Ramondenc Y, Plé G, Plé N. A new series of rod-like conjugated molecules with a pyrazine or a bipyrazine core. Synthesis and light emitting properties. Tetrahedron 2011;67:2287–98.

[13] (a) Cristiano R, Westphal E, Bechtold IH, Bortoluzzi AJ, Gallardo H. Synthesis and optical/thermal properties of low molecular mass V-shaped materials based on 2,3-dicyanopyrazine. Tetrahedron 2007;63:2851–8;
(b) Jaung JY, Matsuoka M, Fukunishi K. Dicyanopyrazine studies. Syntheses and characterization of new bis-styryl fluorescent dyes from DAMN. Dyes Pigm 1998;36:395–405;
(c) Schmitt V, Fischer J, Detert H. Linear and angular distyrylpyrazines with terminal donor groups: synthesis, solvatochromism, and acidochromism of the electronic epectra ISRN Org. Chem. doi:10.5402/2011/589012. (d) Bureš F, Čermáková H, Kulhánek J, Ludwig M, Kuznik W, Kityk IV, et al. Structure-property relationships and nonlinear optical effects in donor-substituted dicyanopyrazine-derived push–pull chromophores with enlarged and varied π-linkers. Eur.J Org Chem; 2012:529–38.

[14] Crosby GA, Demas JN. Measurement of photoluminescence quantum yields – review. J Phys Chem 1971;75:991–1024.

[15] (a) Vanden Eynde JJ, Pascal L, Van Haverbeke Y, Dubois P. Quaternary ammonium salt-assisted synthesis of extended pi-systems from methyldiazines and aromatic aldehydes. Synth Commun 2001;31:3167–73;
(b) Hadad C, Fiol-Petit C, Cornec AS, Dupas G, Ramondenc Y, Plé N. Rod-like conjugated molecules with ethynylene linkage and pyridazine moieties: synthesis and light emitting properties. Heterocycles 2010;81:1445–57;

(c) Jaung JY, Matusoka M, Fukunishi K. Syntheses and properties of new styryl dyes derived from 2,3-dicyano-5-methylpyrazines. Dyes Pigm 1996;31: 141–53;
(d) Hadad C, Achelle S, Garcia-Martinez JC, Rodriguez-Lopez J. 4-Arylvinyl-2,6-(pyridine-2yl)pyrimidines: synthesis and optical properties. J Org Chem 2011; 76:3837–45;
(e) Lipunova GN, Nosova EV, Trashakhova TV, Charushin VN. Azinylarylethenes: synthesis and photophysical and photochemical properties. Russ Chem Rev 2011;80:1115–30.

[16] (a) Mallegol T, Gmouh S, Meziane MAA, Blanchard-Desce M, Mongin O. Practical and efficient synthesis of tris(4-formylphenyl)amine: a key building block in materials chemistry. Synthesis; 2005:1771–4;
(b) Wang YJ, Sheu HS, Lai CK. New star-shaped triarylamines: synthesis, mesomorphic behavior, and photophysical properties. Tetrahedron 2007;63: 1695–705.

[17] (a) Gao Y, Shreeve JM. Quaternization of pyrazine, pyridazine and pyrimidine with alkyl and polyfluoroalkyl halides: formation of low melting salts. Synthesis 2004;7:1072–82;
(b) Duffin GF. The quaternarization of heterocyclic compounds, vol. 3; 1964. p. 1–56.

[18] Myśliwa-Kurdziel B, Solymosi K, Kruk J, Böddi B, Straszalka K. Solvent effects on fluorescence properties of protochlorophyl and its derivatives with various porphyrin side chains. Eur Biophys J 2008;37:1185–93.

[19] See for example. (a) Katan C, Terenziani F, Mongin O, Werts MHV, Porres L, Pons T, et al. Effects of (multi)branching of dipolar chromophores on photophysical properties and two-photon absorption. J Phys Chem A 2005;109: 3024–37;
(b) Lincker F, Kreher D, Attias AJ, Do J, Kim E, Hapiot P, et al. Rodlike fluorescent pi-conjugated 3,3′-bipyridazine ligand: optical, electronic, and complexation properties. Inorg Chem 2010;49:3991–4001.

[20] (a) Nordén B, Kurucsev T. Analysing DNA complexes by circular and linear dichroism. J Mol Recognit 1994;7:141–55;
(b) Dumas B, Bordeau G, Faurel-Paul E, Mahuteau-Betzer F, Saettel N, Bombled M, et al. N-phenyl-carbazole-based two-photon fluorescent probes: strong sequence dependence of the duplex versus quadruplex selectivity. Biochimie 2011;93:1209–18.