Convergent and Practical Synthesis of Fluorescent Triphenylamine Derivatives and Their Localization in Living Cells

Romain Mougeot, Samuel Oger, Marie Auvray, Thibault Gallavardin, Stéphane Leleu, Florence Mahuteau-Betzer, Xavier Franck

Convergent and Practical Synthesis of Fluorescent Triphenylamine Derivatives and Their Localization in Living Cells

Romain Mougeot,[a] Samuel Oger,[a] Marie Auvray,[b],[c] Thibault Gallavardin,[a] Stéphane Leleu,[a]Florence-Mahuteau-Betzer,*[b],[c] Xavier Franck*[a]

[a] Dr. R. Mougeot, Dr. S. Oger, Dr. T. Gallavardin, Dr. S. Leleu, Dr. X. Franck
Normandie Univ, CNRS, INSA Rouen, UNIROUEN, COBRA (UMR 6014 and FR 3038), 76000 Rouen, France
E-mail: xavier.franck@insa-rouen.fr

[b] Ms M. Auvray, Dr. F. Mahuteau-Betzer
Institut Curie, Université PSL, CNRS UMR9187, Inserm U1196
Chemistry and Modeling for the Biology of Cancer
91400 Orsay, France
E-mail: florence.mahuteau@curie.fr

[c] Université Paris-Saclay, CNRS UMR9187, Inserm U1196
Chemistry and Modeling for the Biology of Cancer
91400 Orsay, France

Abstract: In the search for new fluorescent triphenylamine (TP) derivatives, we studied the influence of the position and substitution of diverse heterocyclic substituents. A library of 10 fluorescent triphenylamines bearing either oxazoles or thiazoles and pyridiniums, substituted at different positions has been developed. The approach is based on a convergent C-H activation reaction between pyridine-oxazoles or pyridine-thiazoles and di-iodo triphenylamine. We showed that the nature and substitution pattern of the 5-membered-(oxazole, thiazole) or 6-membered heterocycle (pyridine) has a strong influence on their fluorescence properties and on their localization in living cells as they stain either the nucleus or mitochondria.

Introduction

The design and synthesis of efficient fluorescent probes is still of great interest particularly for cellular imaging. Although some efficient and currently used fluorophores already exist, new fluorophores with optimal photophysical and biochemical properties are still needed.[1,2] The main photophysical requirements are high fluorescence quantum yield, red-shifted absorption/emission wavelengths and large Stokes shift. Once, they fulfill these criteria, the dyes have to be cell-permeant, non-cytotoxic and photostable. They also need to be water soluble and should possess low molecular weight. Finally, to enhance the signal-to-noise ratio, the design of OFF-ON probes is very attractive[3] especially when fluorescence is restored through binding to the cellular target or through reaction with cellular analytes.[4]

In this context, a few years ago, we developed a new series of cationic vinylpyridinium triphenylamine (TP) turn-on probes that stained nuclear DNA in fixed cells.[5] In order to determine structure-activity relationship, we modified the vinyl bond to an oxazole ring.[6] Indeed, the 2,5-di(hetero)aryloxazoles are known for their attractive photophysical properties (high quantum yields, strong absorption) [7,8,9] and the modification of the vinyl bond to a metabolically and chemically more stable heterocycle was also an asset. However, as its vinylpyridinium triphenylamine parent compound (**TP-2Py**), the pyridinium-oxazole TP does not reach nucleus in living cells but labels mitochondria.[10,11] These cationic triphenylamines are delocalized lipophilic cation which are known to be sequestrated in the mitochondria. In addition, diaryloxazoles and push-pull diarylthiazoles display interesting photophysical properties.[12,13]

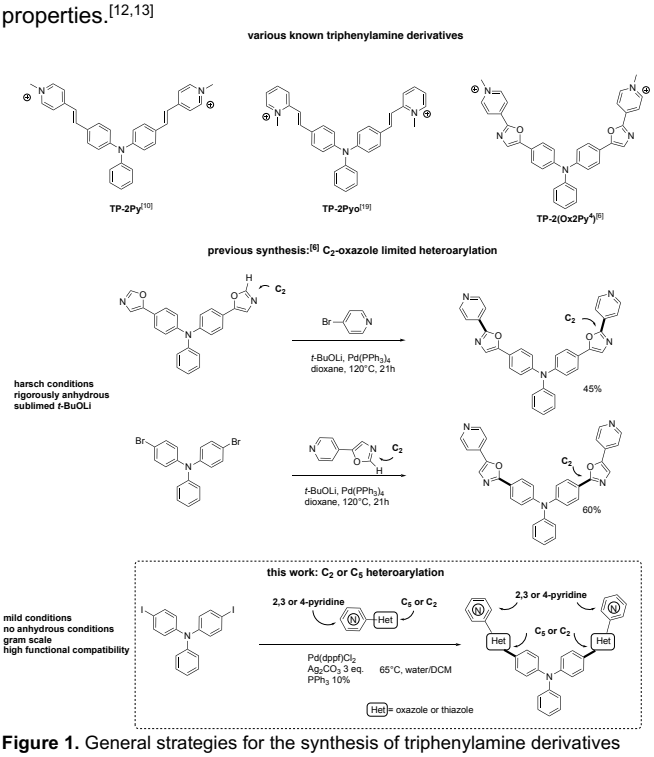


Figure 1. General strategies for the synthesis of triphenylamine derivatives

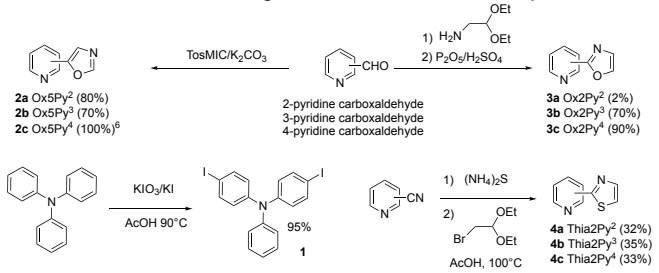
Originally, our synthetic strategy to prepare these pyridinium-oxazole TP was built on a selective functionalization at the C2-oxazole position by C-H activation (Figure 1).[6] Although this method was efficient, it was limited in the order of connection as only C2 functionalization could be achieved; it also required drastic conditions such as high temperature and freshly sublimed *t*-BuOLi. More practical conditions were then necessary to be

developed in order to extend the study of triphenylamine derivatives to other heterocycles and substitution positions, while keeping the synthesis straightforward.
In this paper, we report the design and synthesis of various oxazoyl- and thiazoyl-triphenylamine derivatives in order to complete the structure-activity relationship study. We developed a new convergent synthesis and prepared efficiently 10 oxazoyl- and thiazoyl-TP where the 5-membered heterocycle (oxazole, thiazole) of the pyridine-heterocycle building block is linked at either position 2 or 5 to the triphenylamine core (Figure 1).

The photophysical properties of these novel TP-oxa or thiazoles were investigated by UV-vis absorption and fluorescence both in water and glycerol to study their turn-on behavior. Most of them are red-emissive and display moderate to good fluorescence quantum yields. The most promising fluorescent probes were then evaluated by confocal microscopy in A549 live cells.

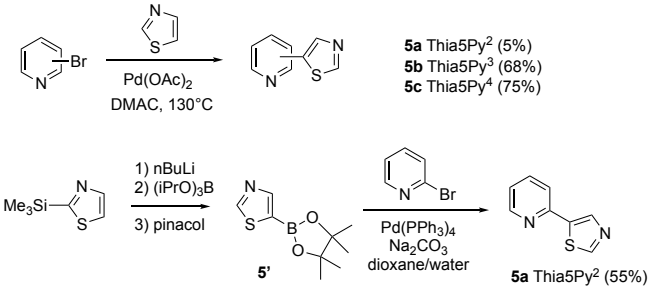
Results and Discussion

The starting materials were prepared using conventional methods (Scheme 1). Di-iodo triphenylamine **1** was prepared by iodination in the presence of KIO_3/KI in acetic acid.[14] 5-(Pyridinyl)oxazole derivatives Ox5Py$^{2\text{-}4}$ **2a-c** were prepared by Van Leusen reaction from 2,3 or 4-pyridinecarboxaldehydes with good yields.
The regioisomeric 2-(pyridinyl)oxazole derivatives Ox2Py$^{2\text{-}4}$ **3a-c** were also prepared from 2,3 or 4-pyridinecarboxaldehydes, *via* their imine and subsequent cyclisation under acidic conditions, in good yields except for Ox2Py^2 **3a** that was obtained in a dramatically low 2% yield; however scaling-up this reaction allowed us to obtain enough material for the next step.



Scheme 1. Synthesis of pyridine-oxazoles and pyridine-thiazoles derivatives

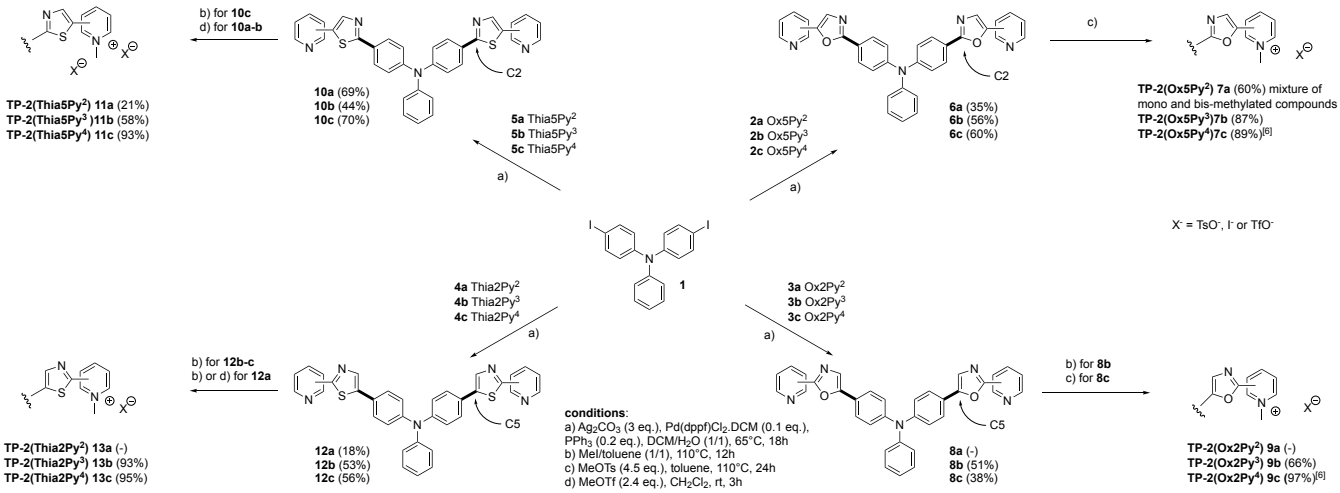
In view of the final 5-thiazole C-H couplings, the 2-(pyridinyl)thiazoles derivatives Thia2Py$^{2\text{-}4}$ **4a-c** were prepared starting from the corresponding 2,3 or 4-cyanopyridines after conversion into thioamides by treatment with $(NH_4)_2S$ followed by alkylation with 2-bromo-1,1-diethoxyethane and cyclization under acidic conditions.
For the final 2-thiazole C-H couplings, the 5-(pyridinyl)thiazole derivatives Thia5Py$^{2\text{-}4}$ **5a-c** were prepared by performing first a 5-selective C-H coupling of 2,3 or 4-bromopyridines with thiazole under Itami's conditions (Scheme 2).[15] This proved to work well for 3-and 4-bromopyridines, yielding Thia5Py^3 **5b** and Thia5Py^4 **5c** with 68 and 75% yield, respectively. However, for 2-bromopyridine, the yield was only 5% for Thia5Py^2 **5a**, therefore we envisaged another strategy using Suzuki cross-coupling. 5-thiophene pinacolboronate **5'** was prepared with 62% yield via metalation of 2-TMS-thiophene following reported procedure.[16]
The resulting boronate was then coupled with 2-bromopyridine to give Thia5Py^2 **5a** with 55% yield.



Scheme 2. Synthesis of Thia5Py$^{2\text{-}4}$ **5a-c**

Having now in hand sets of pyridine-oxazoles and pyridine-thiazoles, their coupling at either position C2 or C5 of the 5-membered heterocycle with di-iodo triphenylamine **1** was studied. We turned our attention to the "on water" CH activation conditions described by Greaney[17,18] because of their mildness and high compatibility with oxazoles and thiazoles (Scheme 3).
We started the 2-oxazole C-H coupling studies between the three Ox5Py$^{2\text{-}4}$ derivatives **2a-c**, where the pyridine ring is substituted by oxazole at positions 2, 3 and 4, and di-odo triphenylamine **1**. When we applied the "on water" conditions to those substrates, we faced irreproducible experiments resulting from important clogging in the sealed tube, probably due to high insolubility of the products that have high molecular weights. To minimize clogging, we increased the amount of dichloromethane from 5% to 50%; leading to purely biphasic conditions. In those conditions, bis-branched triphenylamine derivatives **6a-c** could be obtained in reproducible yields, ranging from 35 to 60%, depending on the position of the pyridine substitution (the 2-substituted pyridine Ox5Py^2 **2a** giving the lower yield). The pyridine rings of **6a-c** where then methylated with methyl tosylate to give the pyridinium salts TP-2(Ox5Py$^{3\text{-}4}$) **7b-c** as red solids with good yields while **7a** proved to be an inseparable mixture of mono and bis-methylated compounds and was therefore not considered for further studies. Application of those conditions to the three Ox2Py$^{2\text{-}4}$ derivatives **3a-c** led to the formation of the bis-branched triphenylamine derivatives **8b-c** with 51 and 38% yield, respectively; no coupling product **8a** could be obtained with Ox2Py^2 **3a** where the pyridine ring is substituted at position 2. This could probably be explained by the spatial arrangement of the heteroatoms of the oxazole-pyridine moiety that could form a pincer able to chelate metals, thus preventing the running of the catalytic cycle. The two pyridine rings of **8b-c** were methylated with either MeOTs (for **8c**, giving TP-2(Ox2Py^4) **9c** almost quantitatively) or MeI (for **8b**, giving TP-2(Ox2Py^3) **9b** with 66% yield) as MeOTs did not work for the latter.
After oxazole series was achieved, the 2- or 5-Thiazole CH di-functionalization was studied. The coupling of Thia5Py$^{2\text{-}4}$ **5a-c** with di-iodo triphenylamine **1** under the same biphasic conditions led to the bis-branched triphenylamine derivatives **10a-c** with 69, 44 and 70% yield, respectively. Methylation of the two pyridine moieties in **10a-c** was achieved by using MeI for **10a** whereas **10b-c** required MeOTf. TP-2(Thia5Py$^{2\text{-}4}$) **11a-c** were then obtained with yields ranging from 21 to 93%, the lower yield being obtained for the 2-substituted pyridine TP-2(Thia5Py^2) **11a**.
The coupling of Thia2Py$^{2\text{-}4}$ **4a-c** with di-iodo triphenylamine **1** under the same conditions led to the bis-branched triphenylamine derivatives **12a-c** with 18, 53 and 56% yields, respectively. Again, the lower yield is obtained when pyridine is substituted in position 2 (Thia2Py^2, **4a**). Methylation of the two pyridine moieties in **12a-**

c was achieved by using MeI for **12b-c** yielding TP-2(Thia2Py^3) **13b** and TP-2(Thia2Py^4) **13c** with excellent yields; however, reaction of **12a** with either MeI or MeOTf, led only to degradation products.



Scheme 3. Convergent synthesis of triphenylamine derivatives.

Photophysical properties

Photophysical properties of the triphenylamines series **7**,**9**,**11** and **13** were studied. Absorbances were measured in water (Figure 2); as expected, the absorption maximum depends strongly on the substitution of the pyridinium, with red shifted bands in the case of *para* methyl pyridinium by comparison with *ortho*- and *meta*-regioisomers. A more singular observation was the effect of oxazole and thiazole orientation. Compounds **7b-c** and **11a-c** featured sharper bands than compounds **9b-c** and **13b-c** respectively.

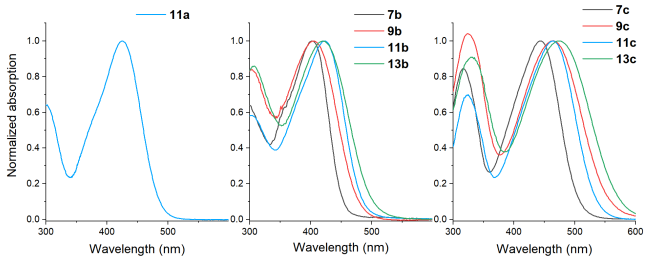


Figure 2. Absorption of triphenylamines in water sorted by pyridinium substitution, *ortho* compounds (left), *meta* compounds (middle), *para* compounds (right).

There was no measurable fluorescence in water, therefore measurements were carried out in highly viscous glycerol (Table 1), as it is expected that movement restriction is the main mechanism of fluorescence activation upon binding to DNA. Some compounds became fluorescent in glycerol (1415 cP at 20°C). Viscosity-fluorescence dependence has been studied for the most fluorescent compounds **7c** and **11a** (Figure S1-S2) confirming that fluorescence turn-on of these compounds is related to molecular mobility restriction and not to polarity effects. As expected, the fluorescence wavelength followed the same trend as the absorption with compounds *ortho*-**a** and *meta*-**b** emitting below 600 nm and more conjugated compounds *para*-**c** between 650 and 665 nm. (Figure 3). There is a blue-shift in emission of **11a** compared to its parent compound **TP-2Py** (574 nm *versus* 633 nm) while there is a slight red-shift for **11c** compared to **TP-Pyo** (665 versus 649 nm).

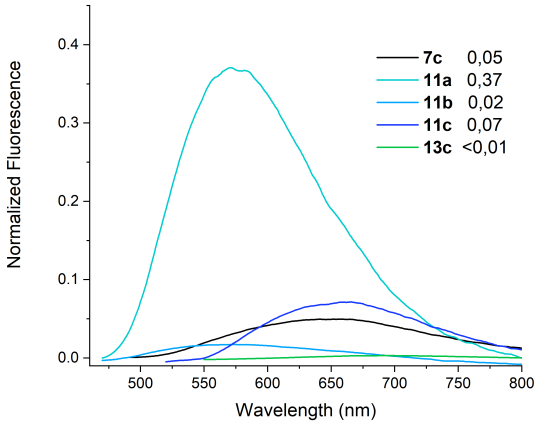


Figure 3. Fluorescence of triphenylamines in glycerol. The amplitude corresponds to the measured fluorescence quantum yield.

Compounds of series **11** were by far the most fluorescent (Table 1) with *ortho* methyl-pyridinium **11a** largely above the others (fluorescence quantum yield φF = 0,37). The same regioisomers but containing an oxazole in place of thiazole (series **7**) are the second most fluorescent (φF up to 0.05 for *para* methyl-pyridinium **7c**). On the contrary, series **9** and **13** are not or poorly fluorescent, showing that orientation of the thiazole/oxazole ring is a key parameter for fluorescence (in glycerol). Interestingly, the most conjugated compounds *i.e.* molecules **c** bearing pyridinium in *para* position are less fluorescent. This suggests that conjugation is detrimental to fluorescence as strong internal charge transfers at excited state led to fluorescence quenching. Finally, *meta* compounds **b** are not or only slightly fluorescent (2% for **11b**). The position of the nitrogen on the pyridine moieties is therefore determinant for the photophysical properties of the dyes: i) the absorption wavelengths are red-shifted for the *para* series compared to the others; ii) the fluorescence quantum yield of the probes with the same central core respects the following order φFa> φFb>> φFc. It is interesting to note that compound **11a** is brighter than its parent compound **TP-2Pyo** as it displays both the highest fluorescence quantum yields and highest molar extinction

coefficient. Even though its emission is blue-shifted compared to **TP-2Pyo**, compound **11a** is a promising dye for cellular imaging.

Table 1. Spectroscopic data.

Compounds	$\lambda_{abs,\ max}$ [a] (ε) [b] water	$\lambda_{em,\ max}$ [a] glycerol	φF [c] glycerol
7b	404 (42 000)	nd	<0.005
7c	444 (41 000)	650	0.05
9b	405 (43 000)	nd	<0.005
9c	465 (41 000)	nd	<0.005
11a	425 (49 000)	574	0.37
11b	420 (57 000)	567	0.02
11c	465 (63 000)	665	0.07
13b	419 (51 000)	nd	<0.005
13c	474 (51 000)	nd	<0.005
TP-2Pyo	478 (41 500)	633	0.23
TP-2Py	509 (31 400)	649	0.11

[a] maximum absoprtion or emission wavelength in nm. [b] molar extinction coefficient $L.mol^{-1}.cm^{-1}$. [c] fluorescence quantum yield determined with integration sphere. nd: not detected

Cellular imaging

The live-cell imaging of the three fluorescent probes **7c**, **11a** and **11c** was performed. First, the biocompatibility of TP dyes was evaluated in A549 cells *via* MTT assay (Figure S3). The probes showed no significant effects on cell viability at 10 µM after 24h of incubation, and can be used for live-cell imaging. After incubation at 2 µM in A549 live cells for 2 hours, **11a** displayed cytoplasmic staining (Figure 4). Colocalization experiments with **11a** and Mitotracker® Deep Red confirmed its mitochondrial staining (Figure 5). Excitation at 633 nm led to emission of Mitotracker® Deep Red whereas excitation at 405 nm led to emission of **11a**. Spatial colocalization was analyzed using Van Steensel's method (see Figure S6). This is in accordance with the behavior of vinyltriphenylamines**TP-2Py** and its *o*-pyridinium analogue **TP-2Pyo** reported by our group.[10,19] Indeed, these DNA ligands stain nuclear DNA in fixed cells but mitochondria in live cells as these lipophilic cations are sequestrated in mitochondria before reaching the nucleus. At the opposite, compounds **7c** and **11c** displayed a bright nuclear labeling and a diffuse cytoplasmic staining in live cells. As these compounds are not cytotoxic, this behavior is probably due to photocytotoxicity. In fact, this pattern is similar to the staining of vinyltriphenylamine dyes under continuous illumination.[10,19,20] Indeed, under photoactivation, vinyl TP dyes triggered apoptosis and this phenomenon could be monitored by the re-localization of the dyes in the nucleus. This similar staining suggests that compounds **7c** and **11c** are prone to photoactivation even with a shorter illumination time. Transmission images showed concomitant formation of membrane blebs, amorphological hall-mark of apoptosis (Figure S5). The *o*-pyridinium analogue **11a** is less sensitive to photoactivation as it did not relocalize under the same illumination conditions. This susceptibility to photoactivation seems to be higher for *p*-pyridinium series than for *o*-pyridinium series as we already noticed in a previous study with vinylpyridinium derivatives.[19] Indeed, photoactivation of **TP-2Pyo** required longer irradiation duration than its *p*-pyridinium **TP-2Py** analogue. Thus, as for compounds **TP-2Py** and **TP-2Pyo**, a prolonged illumination is necessary to observe this phenomenon for **11a**. Therefore, they can be imaged without any photocytotoxicity in live cells. Conversely, dyes **7c** and **11c** quickly led to phototoxicity making them good candidates for photodynamic therapy.

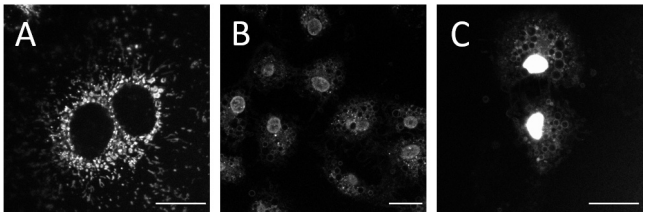


Figure 4. Live-cell imaging of **11a** (A), **7c** (B), and **11c** (C) (2 µM) after incubation for 2h in A549 cells (λ_{exc} = 405 nm; emission slits settings: 500-700 nm; Scale bar: 20µm).

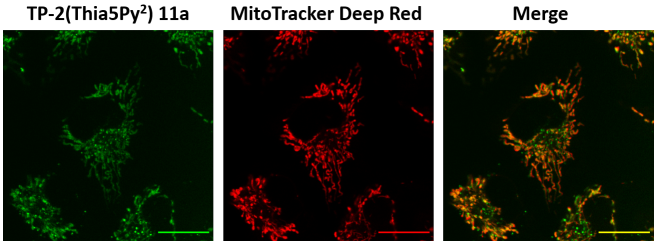


Figure 5. Co-localization experiments with **11a** (first incubation: 2 µM, 2h; λ_{exc} = 405 nm, emission slits settings: 500-700 nm) and MitoTracker® Deep Red (second incubation: 100 nM, 15 min; $\lambda_{exc.}$ = 633 nm, emission slits settings: 650-700 nm) in A549 cells. Scale bar: 20 µm. The green spots that do not colocalize might correspond to the labeling of late endosomes.[20]

Conclusion

We have developed an efficient and convergent strategy to access diverse triphenylamine derivatives. The strategy is built on heteroarylation by C-H activation under slightly modified Greaney's conditions, allowing us to obtain a library of 10 fluorescent triphenylamines bearing either oxazoles or thiazoles and pyridinium, substituted at different positions. As their parent vinylpyridinium triphenylamines, these compounds are not fluorescent in water and some of them restore their fluorescence in glycerol. In fact, their emission properties in glycerol depend both on the orientation of the pyridinium moiety and the heterocyclic connection. All the *m*-pyridinium Py^3 are not fluorescent, while the *o*-pyridinium Py^2 series is the most promising compounds with fluorescence quantum yields of 0.37 for **11a**. These studies also revealed that the orientation of the thiazole or oxazole ring was determinant for the photophysical properties of these triphenylamine derivatives. When the thiazole/oxazole ring is tethered to the triphenylamine in position 2, the compounds are fluorescent in glycerol (series **7** and **11**) whereas the connection in position 5 inhibits the fluorescence (series **9** and **13**). Therefore, we evaluated the 3 most promising compounds **7c**, **11a** and **11c** through live-cell imaging. The *o*-pyridinium compound **11a** is bright mitochondrial stainer as its

parent compound **TP-2Pyo**; whereas the para derivatives **7c** and **11c** could be promising PDT candidates as they quickly undergo photoactivation.

Experimental Section

General procedure 1 for preparation of Ox5Py^n 2a-c

A mixture of pyridine carboxaldehyde (1 eq.), toluenesulfonylmethyl isocyanide (3.3 eq.) and K_2CO_3 (4 eq.) in methanol (1 M) was heated at 80 °C and stirred for 2 hours. The crude mixture was concentrated to dryness and the resulting residue was diluted with DCM. The organic layer was washed with water, dried over $MgSO_4$ and concentrated under vacuum. The crude residue was dissolved in a minimal amount of DCM and a large quantity of pentane was added to afford a precipitate which was filtered to obtain the pure products **2** as a yellow foam.

General procedure 2 for preparation of Ox2Py^n 3a-c

Pyridine carboxaldehyde (1 eq.) and 2,2-diethoxyethanamine (1 eq.) were dissolved in toluene (0.15 M). The reaction mixture was heated to 110 °C and stirred for 12 hours. The crude mixture was concentrated to dryness to afford the crude material which was added portion wise to a first solution containing concentrated H_2SO_4 (0.7 M) at 0 °C, until the full dissolution of the crude mixture. In another round bottom flask, P_2O_5 (3 eq.) was added portion wise at 0 °C to a second solution containing concentrated H_2SO_4 (0.7 M), until the full dissolution of P_2O_5. Then, the first solution was added dropwise to the second solution at 0 °C. The resulting mixture was stirred at 0°C during 30 minutes and then heated at 100 °C for 2 hours. The reaction mixture was poured onto crushed ice, neutralized with solid K_2CO_3 and extracted with DCM. The organic layers were dried over $MgSO_4$ and concentrated under vacuum. The resulting crude was purified by flash chromatography (cyclohexane/EtOAc 80/20 to 50/50) to afford the desired products **3** as a white powder.

General procedure 3 for preparation of Thia2Py^n 4a-c

A solution of pyridine carbonitrile (1 eq.) and $(NH_4)_2S$ (1 eq.) in MeOH (0.5 M) was stirred at room temperature for 18 hours. The resulting mixture was washed with water and extracted with EtOAc. The organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude mixture was filtered on silica gel (EtOAc 100 %) to afford the intermediate thioamide **4'** as a yellow powder. A solution of thioamide (1 eq.) and 2-bromo-1,1-diethoxyethane (1 eq.) in acetic acid (1 M) was stirred at 100°C for 16 hours. The resulting mixture was washed with saturated $NaHCO_3$ and was extracted with EtOAc. The organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude mixture was purified by flash chromatography (silica gel, cyclohexane/EtOAc, 100/0 to 70/30) to afford the desired product **4** as a yellow powder.

5-(Pyridin-2-yl)thiazole (5a).

To a stirred solution of 2-trimethylsilylthiazole (1 eq.) in dry THF (0.1 M) cooled to -78°C, was slowly added at -78 °C *n*-BuLi (1.2 eq.). After 1 hour at -78°C triisopropyl borate (1.2 eq.) was added to the mixture at -78 °C and stirred at this temperature for 1.5 hours. The mixture was then warm to room temperature and stirred for an additional 1.5 hours. 2,3-dimethylbutane-2,3-diol (1.1 eq.) was then added to the mixture and stirred during 15 minutes. Then, glacial acetic acid was added until pH=5 and stirred at room temperature for 45 minutes. The resulting mixture was diluted with ether, filtered and concentrated under vacuum. To remove the excess of acetic acid, the mixture was diluted with cyclohexane and concentrated under vacuum three times. The resulting crude was then triturated with *n*-hexane and the obtained precipitate was filtered of to afford the desired boronic acid **5'.** To a mixture of 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazole **5'** (2 eq.), 2-bromopyridine (1 eq.) in a mixture of dioxane and water (3/1, 0.06 M) were added Na_2CO_3 (2.5 eq.) and $Pd(PPh_3)_4$ (5 mol%). The reaction mixture was heated at 80 °C and the consumption of 2-bromopyridine was monitored by TLC. The reaction mixture was then diluted with EtOAc and filtered through a pad of celite. The filtrate was washed with brine and the organic layer was dried over $MgSO_4$, filtered, and evaporated under vacuum. The crude product was purified by flash chromatography (silica gel, (cyclohexane/EtOAc 100/0 to 70/30) to afford the desired product **5a**.

5-(Pyridin-3-yl)thiazole (5b)

$Pd(OAc)_2$ (5 mol%), KOAc (2 eq.), 3-bromopyridine (1 eq.), thiazole (2 eq.), and DMAc (0.4 M) were added in a sealed tube. The reaction mixture was then stirred at 130°C for 20 hours. After cooling the reaction mixture to room temperature, the resulting mixture was diluted with EtOAC then washed once with NaOH (1 M), twice with water. The organic layer was dried over $MgSO_4$ and concentrated under vacuum. The crude mixture was purified by flash chromatography (silica gel, 60/40 to 40/60 Cyclohexane/EtOAc) to afford desired product **5b**.

5-(Pyridin-4-yl)thiazole (5c)

$Pd(OAc)_2$ (5 mol%), KOAc (4 eq.), 4-bromopyridinium chloride (1 eq.), thiazole (2 eq.), and DMAc (0.4 M) were added in a sealed tube. The reaction mixture was then stirred at 130 °C for 20 hours. After cooling the reaction mixture to room temperature, the resulting mixture was extracted once with NaOH (1 M), twice with water and washed with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated under vacuum. The crude mixture was purified by flash chromatography (silica gel, 100/0 to 70/30 Cyclohexane/EtOAc) to afford the desired product **5c**.

General procedure 4 for the preparation of 6, 8, 10, 12

1 (1 eq.), **2** (**3**, **4** or **5**) (2 eq.), PPh_3 (0.2 eq.), $Pd(dppf)Cl_2$.DCM (0.1 eq.) and Ag_2CO_3 (3 eq.) were introduced in a sealed tube. Then an equal amount of DCM and water (0.5 M) were added in a sealed tube and heated to 65 °C during 18 hours. The resulting crude was washed with NaOH 1 M and extracted with DCM. The organic layers were dried over $MgSO_4$ and concentrated under vacuum. The resulting crude was purified by flash chromatography (silica gel DCM/MeOH, 100/0 to 90/10) to afford the desired products as a powder.

General procedure 5 fort the methylation using MeI

8b, (**10c**, **12b** or **12c**) (1 eq.) was dissolved in an equal amount of toluene and MeI (0.1 M) and heated at 110°C until completion monitored by HPLC. The crude mixture was concentrated under vacuum. The red precipitate was washed with toluene and dried under vacuum. The products were obtained as a red powder.

General procedure 6 fort the methylation using MeOTs

A suspension of compound **6a** (or **6b**) (1 eq.) and methyl tosylate (4.5 eq.) in toluene (0.1 M) was stirred for 12 hours at reflux. After cooling down to room temperature, the reaction mixture was washed with toluene and diethyl ether to afford **7a** (mixture of mono and bis-methylated products) or **7b** as a red solid.

General procedure 7 fort the methylation using MeOTf

A suspension of compound **10a** or **10b** (1 eq.) and methyl triflate (2.4 eq.) in DCM (1 M) was stirred for 3 hours at room temperature. The orange precipitate was washed with DCM three times and dried under vacuum to afford **11a** or **11b** as red solids.

Acknowledgements

This work was partially supported by Normandie Université (NU), the Région Normandie, the Centre National de la Recherche Scientifique (CNRS), University of Rouen Normandy (URN), INSA Rouen Normandy, European Regional Development Fund (ERDF), Carnot Institute I2C, Labex SynOrg (ANR-11-LABX-0029) for financial support to R.M and the graduate school for research XL-Chem (ANR-18-EURE-0020 XL CHEM). We gratefully thank Mr. Thomas Letellier for the synthesis of some thiazole derivatives. We are grateful to Patricia Martel (University of Rouen Normandy) for IR analyses, and to Albert Marcual (CNRS) for HRMS analyses. The authors greatly acknowledge the Multimodal Imaging Center-Light Microscopy Facility (Institut Curie, CNRS UMS2016, Inserm US43, Université Paris-Saclay) particularly Laetitia Besse and Dr. Marie-Noëlle Soler for their technical assistance in the use of the Leica confocal SP5 system. We thank Dr Marie-Paule Teulade-Fichou for fruitful discussions.

Keywords: C-H activation • Imaging agents • Oxazole • Thiazole • Triphenylamine

[1] P. Gao, W. Pan, N. Li, B. Tang. *Chem. Sci.*, **2019**, *10*, 6035–6071

[2] H. Zhu, J. Fan, J. Du, X. Peng. *Acc. Chem. Res.* **2016**, *49*, 2115−2126

[3] A. S. Klymchenko. *Acc. Chem. Res.* **2017**, *50*, 366−375

[4] K. P. Carter, A. M. Young, A. E. Palmer. *Chem. Rev.* **2014**, *114*, 4564−4601

[5] C. Allain, F. Schmidt, R. Lartia, G. Bordeau, C. Fiorini-Debuisschert, F. Charra, P. Tauc, M.-P. Teulade-Fichou. *ChemBioChem*, **2007**, *8*, 424 – 433.

[6] B. Dumat, E. Faurel-Paul, P. Fornarelli, N. Saettel, G. Metgé, C. Fiorini-Debuisschert, F. Charra, F. Mahuteau-Betzer, M.-P. Teulade-Fichou. *Org. Biomol. Chem.*, **2016**, *14*, 358–370.

[7] Q. Wang, D S. Lawrence. *J. Am. Chem. Soc*. **2005**, *127*, 7684-7685.

[8] J. Min, J. W. Lee, Y-H Ahn, Y.-T. Chang. *J. Comb. Chem.* **2007**, *9,* 1079–1083

[9] S. Charier, O. Ruel, J.-B. Baudin, D. Alcor, J.-F. Allemand, A. Meglio, L. Jullien, Bernard Valeur. *Chem. Eur. J*. **2006**, *12*, 1097-1113.

[10] R. Chennoufi, H. Bougherara, N. Gagey-Eilstein, B. Dumat, E. Henry, F. Subra, F. Mahuteau-Betzer, P. Tauc, M.-P. Teulade-Fichou, E. Deprez. *Chem.Commun*.,**2015**,*51*,14881—14884.

[11] P. Tauc, E. Deprez, H. Bougherara, N. Gagey, M.P. Teulade-Fichou, F. Mahuteau-Betzer, B. Dumat. WO2014044757

[12] J. Shikuma, A. Mori, K. Masui, R. Matsuura, A. Sekiguchi, H. Ikegami, M. Kawamoto, T. Ikeda. *Chem. Asian J.* **2007**, *2*, 301 – 305

[13] A. Mori, A. Sekiguchi, K. Masui, T. Shimada, M. Horie, K. Osakada, M. Kawamoto, T. Ikeda. *J. Am. Chem. Soc*. **2003**, *125*, 1700-1701.

[14] M. Grigoras. A. M. Catargiu, T. Ivan, L. Vacareanu, B. Minaev, E. Stromylo. *Dyes and Pigments* **2015**, *113*, 227-238.

[15] S. Tani, T. N. Uehara, J. Yamaguchia, K. Itami, *Chem. Sci.* **2014**, *5*, 123–135

[16] N. Primas, A. Bouillon, J.-C. Lancelot, S. Rault, *Tetrahedron*, **2009**, *65*, 6348–6353

[17] S. A. Ohnmacht, P. Mamone, A. J. Culshaw, M. F. Greaney, *Chem. Commun.*, **2008**, 1241–1243

[18] G. L. Turner, J. A. Morris, M. F. Greaney, *Angew. Chem. Int. Ed.* **2007**, *46*, 7996 –8000.

[19] R. Chennoufi, N.-D. Trinh, F. Simon, G. Bordeau, D. Naud-Martin, A. Moussaron, B. Cinquin, H. Bougherara, B. Rambaud, P. Tauc, C. Frochot, M.-P. Teulade-Fichou, F. Mahuteau-Betzer, E. Deprez. *Sci. Rep*. **2020**, *10*, 6881.

[20] R. Chennoufi, H. Bougherara, N. Gagey-Eilstein, B. Dumat, E. Henry, F. Subra, S. Bury-Moné, F. Mahuteau-Betzer, P. Tauc, M.-P. Teulade-Fichou, E. Deprez. *Sci. Rep.* **2016**, *6*, 21.