Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Live Cell Cytoplasm Staining and Selective Labeling of Intracellular Proteins by Non-Toxic Cell-Permeant Thiophene Fluorophores

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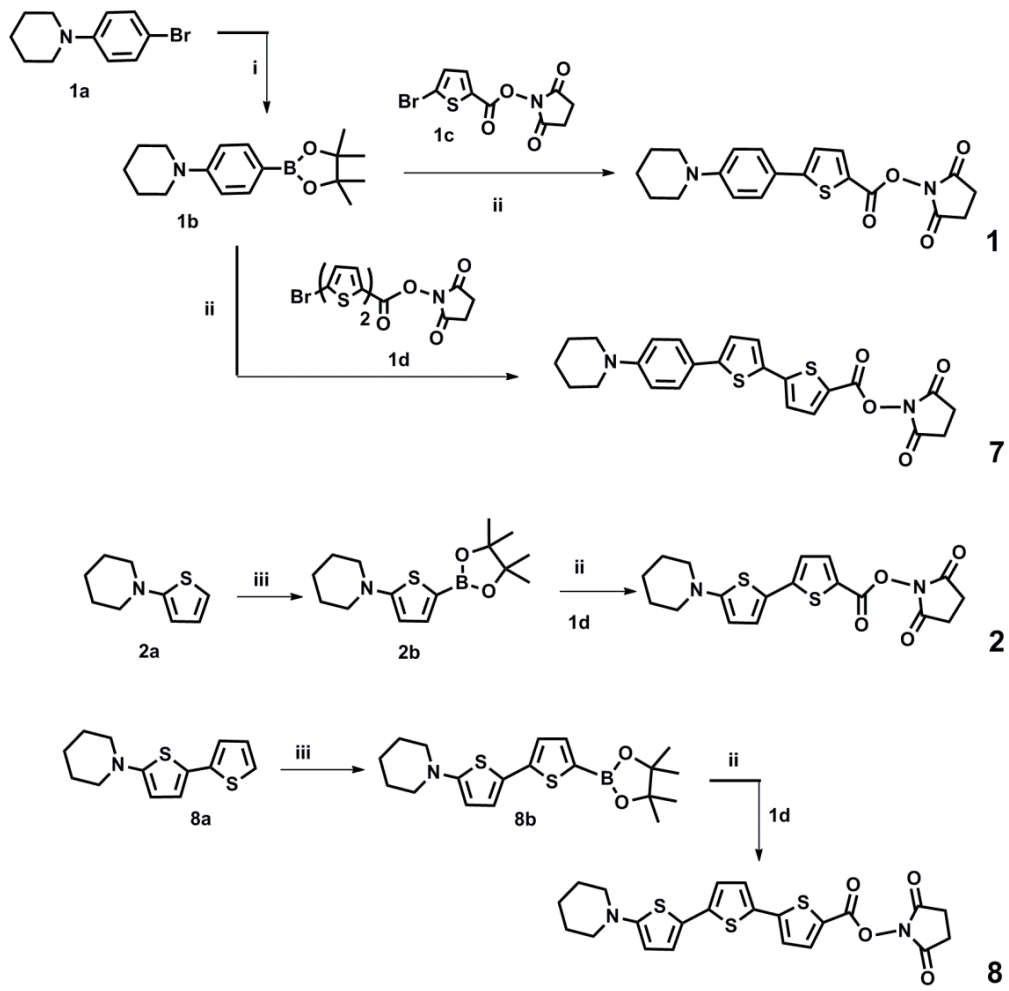
SUPPLEMENTARY INFORMATION

I. SYNTHESIS AND CHARACTERIZATION

General. Organic solvents were dried by standard procedures. Lithiation and cross coupling reactions were carried out under nitrogen atmosphere. Glass columns of different sizes were used for silica gel chromatographies (particle sizes 0.040-0.063 mm, Merck) or florisil (100-200 mesh, Aldrich). Bis(pinacolato)diboron, namely 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, was purchased from Alfa Aesar GmbH & Co KG; sodium bicarbonate from Sigma-Aldrich Co; 1,1′-bis(diphenylphosphino)ferrocene palladium(II)chloride dichloromethane complex ($PdCl_2$dppf), *n*-butyllithium 2.5 M solution in hexane from Acros Organics. All reagents and solvents were used as received. Microwave irradiation was achieved in a Milestone Microsynth Labstation operating at 2450 MHz equipped with pressure and temperature sensors. Reactions were performed in 10 mL glass vessels sealed with a septum. Microwave irradiation was power controlled (maximum power input: 100 W) and the samples were irradiated with the required power output to achieve the desired temperature. Melting points were obtained on Kofler bank apparatus and are uncorrected. All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-400 spectrometer equipped with a 5-mm probe. Chemical shifts are referenced to TMS. Mass spectra were collected on a Finningan Mat GCQ spectrometer.

Synthesis of compounds 1-2 and 7-8



Reagents and conditions: i) $PdCl_2$(dppf), bis(pinacolato)diboron, $NaHCO_3$, THF/H_2O, MW, 10 min, 80°C; ii) $PdCl_2$(dppf), 3 mL DMF/H_2O 2:1 v/v, 1 eq **1c** or **1d**, 3 eq **1b**, or **2b** or **8b**, MW 10 min, 80°C; iii) BuLi 2.5 M, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, THF, -70°C.

Compounds **1c** and **1d** have already been described.[6a]

***General procedure for the synthesis of compounds* 1-2** and **7-8.** A mixture of monobromo derivatives **1c** or **1d** (0.38 g, 1 mmol), thienylboronic ester (**1b**, **2b**, **8b**) (3 mmol), Pd $(dppf)_2Cl_2$ (0.05 mmol) and $NaHCO_3$ (2 mmol) in DMF/H_2O (2:1, 3 mL) was irradiated with microwaves at 80°C for 10 min. The reaction mixture was brought to room temperature and the solvent was evaporated under reduced pressure. All compounds were isolated by flash chromatography on florisil.

***2,5-dioxopyrrolidin-1-yl 5-(4-(piperidin-1-yl)phenyl)-thiophene-2-carboxylate* (1)**
→ Hexane : AcOEt : CH_2Cl_2 70:20:10Yield 80%. Yellow pale solid; mp>230°C; EI-MS *m/z* 384; absorption maximum, 390 nm (ε 28423 $cm^{-1}M^{-1}$), emission maximum, 513 nm in CH_2Cl_2; ^1H NMR

(CD_2Cl_2, ppm) δ 7.95 (d, ^3J=4.0Hz, 1H), 7.58 (d, J=8.8Hz, 2H), 7.29 (d, ^3J=4.0Hz, 1H), 6.97 (s, 2H), 3.30 (t, 4H), 2.88 (s, 4H), 1.68 (m, 6H); ^{1}H NMR (CD_3COCD_3, ppm) δ 8.00 (d, ^3J=4.0Hz, 1H), 7.66 (d, J=8.8Hz, 2H), 7.48 (d, ^3J=4.0Hz, 1H), 7.04 (d, J=8.8Hz, 2H), 3.30 (t, 4H), 2.95 (s, 4H), 1.67 (m, 6H); ^{13}C NMR (CD_2Cl_2, ppm) δ 169.4, 157.4, 137.9, 137.8, 127.4, 127.3, 122.2, 115.3, 49.3, 25.7, 25.65, 25.6, 25.4, 24.2.

***2,5-dioxopyrrolidin-1-yl 5' –(piperidin-1-yl)-2,2'-bithiophene-5-carboxylate* (2)**

→ Hexane : AcOEt : CH_2Cl_2 70:20:10. Yield 85%. Yellow solid; mp>230°C; EI-MS *m/z* 390; absorption maximum, 440 nm (ε 28423 $cm^{-1}M^{-1}$), emission maximum, 544 nm in CH_2Cl_2; ^{1}H NMR ($CDCl_3$, TMS/ppm) δ 7.84 (d, ^3J=4.4Hz, 1H), 7.09 (d, J=4.0Hz, 1H), 6.94 (d, ^3J=4.0Hz, 1H), 5.98 (d, J=4.4Hz, 1H), 3.20 (t, 4H), 2.88 (s, 4H), 1.72 (m, 4H), 1.60 (m, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 169.3, 161.6, 157.2, 149.9, 137.8, 127.0, 121.0, 120.5, 119.9, 104.5, 51.7, 25.6, 25.0, 23.6.

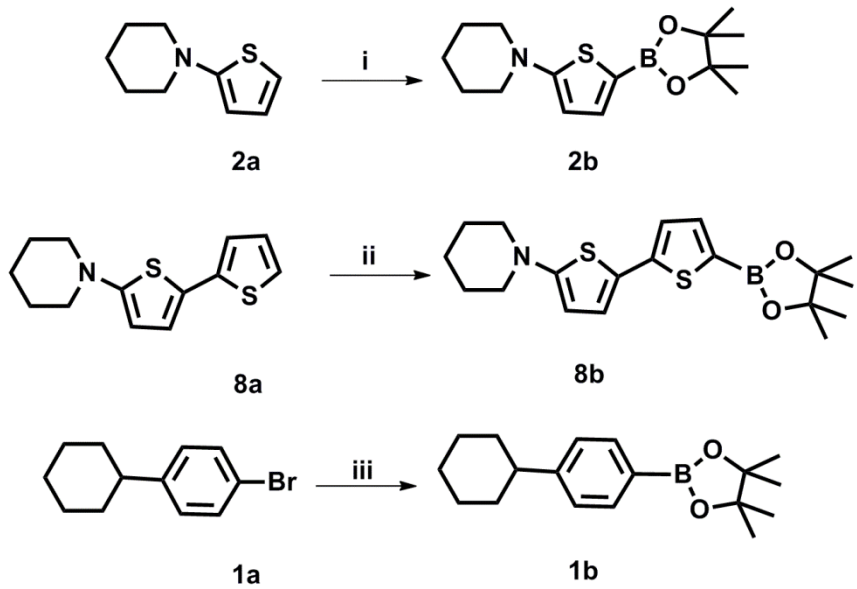
***2,5-dioxopyrrolidin-1-yl 5'-(4-(piperidin-1-yl)phenyl)-2,2'-bithiophene-5-carboxylate* (7)**

→ Hexane : AcOEt : CH_2Cl_2 60:25:15Yield 70%. Yellow solid; mp>230°C; EI-MS *m/z* 466; absorption maximum, 421 nm (ε 59869 $cm^{-1}M^{-1}$), emission maximum, 602 nm in CH_2Cl_2; ^{1}H NMR (CD_2Cl_2, ppm) δ 7.94 (d, ^3J=4.0Hz, 1H), 7.51 (d, J=8.8Hz, 2H), 7.35 (d, ^3J=3.6Hz, 1H), 7.26 (d, ^3J=3.6Hz, 1H), 7.18 (d, ^3J=4.0Hz, 1H), 6.93 (d, J=8.8Hz, 2H), 3.25 (t, 4H), 2.88 (s, 4H), 1.70 (m, 4H), 1.62 (m, 2H); ^{13}C NMR (CD_2Cl_2, ppm) δ 169.5, 157.4, 152.2, 148.5, 147.4, 137.8, 132.7, 127.7, 126.8, 123.9, 123.4, 122.4, 121.0, 115.8, 49.8, 26.4, 25.8, 24.5.

***2,5-dioxopyrrolidin-1-yl 5' –(piperidin-1-yl)-(2,2';5',2'')-terthiophene-5-carboxylate* (8)**

→ Hexane : AcOEt : CH_2Cl_2 60:25:15. Yield 60%. Orange; mp>230°C; EI-MS *m/z* 472; absorption maximum, 454 nm (ε 19256 $cm^{-1}M^{-1}$), emission maximum, 649 nm in CH_2Cl_2; ^{1}H NMR (CD_2Cl_2, ppm) δ 7.89 (d, ^3J=4.0Hz, 1H), 7.20 (d, ^3J=4.0Hz, 1H), 7.13 (d, ^3J=4.0Hz, 1H), 6.94 (d, ^3J=4.0Hz, 1H), 6.88 (d, ^3J=4.0Hz, 1H), 5.98 (d, ^3J=4.0Hz, 1H), 3.20 (t, 4H), 2.89 (s, 4H), 1.72 (m, 4H), 1.59 (m, 2H); ^{13}C NMR ($CDCl_3$, ppm) δ 169.2, 160.2, 157.2, 148.2, 141.0, 137.7, 131.4, 127.1, 124.6, 123.3, 123.0, 121.8, 121.3, 104.6, 52.0, 25.6, 25.1, 23.7.

Synthesis of boronic esters 2b, 8b, 1b



Reagents and conditions: i, ii) BuLi 2.5 M, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, THF, -70°C; iii) $PdCl_2$(dppf), bis(pinacolato)diboron, $NaHCO_3$, THF/H_2O, MW, 10 min, 80°C.

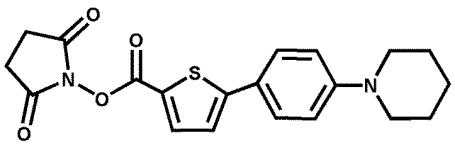
General procedure for the syntesis of compound 2b, 1b and 8b. To a stirred solution of **2a**, **8a** and **1a** (1 mmol) in anhydrous THF (10 mL) at –70°C, BuLi (2.5M in hexane) (1.2 mmol) was slowly added. The mixture was allowed to react at this temperature for 1 h, then at room temperature for an additional hour. After cooling at –70°C, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.2 mmol) was added and the resulting mixture was allowed to warm to room temperature and stirred for 12 h. The product was extracted with CH_2Cl_2 (2x100 mL). The combined organic phases were dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure.

1-(5-4,4,5,5-tretramethyl-1,3,2-dioxaborolan-2-yl)thiophen-2-yl)piperidine **(2b)**
Yield 99%. Dark-green solid; EI-MS *m/z* 293; ^{1}H NMR ($CDCl_3$, ppm) δ 7.37 (d, ^3J=4.0Hz, 1H), 6.13 (d, ^3J=4.0Hz, 1H), 3.20 (t, 4H), 1.69 (m, 4H), 1.56 (m, 2H) 1.31 (s, 12H).

1-(5-(4,4,5,5-tretramethyl-1,3,2-dioxaborolan-2-yl)-2,2'-bithiophen-5-yl)piperidine **(8b)**
Dark-green solid. ^{1}H NMR of the crude product showed a mixture of **8b**: **7** (starting material) in the ratio 50:50. The crude was utilized as obtained for the last synthetic step. ^{1}H NMR ($CDCl_3$, ppm) δ 7.46 (d, ^3J=3.6Hz, 1H), 7.00 (d, ^3J=3.6Hz, 1H), 6.94 (m, 2H), 3.14 (t, 4H), 1.71 (m, 4H), 1.58 (m, 2H) 1.33 (s, 12H).

1-(4-(4,4,5,5-tretramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)piperidine **(1b)**
A mixture of compound **1a** (1 mmol), bis(pinacolato)diboron (1.2 mmol), $PdCl_2$(dppf) (0.05 mmol), $NaHCO_3$ (2 mmol) in THF/water 2:1 (3 mL) was irradiated with microwaves at 80°C. The reaction mixture was brought to room temperature and the solvent was evaporated under reduced pressure. the crude product which did not need further purification. Yield 90%. Dark white solid; EI-MS *m/z* 287; ^{1}H NMR ($CDCl_3$, ppm) δ 7.69 (d, *J*=8.8Hz, 2H), 6.87 (d, *J*=8.8Hz, 2H), 3.25 (t, 2H), 1.67 (m, 3H), 1.60 (m, 2H), 1.32 (s, 12H)

II. ^{1}H AND ^{13}C NMR SPECTRA



1

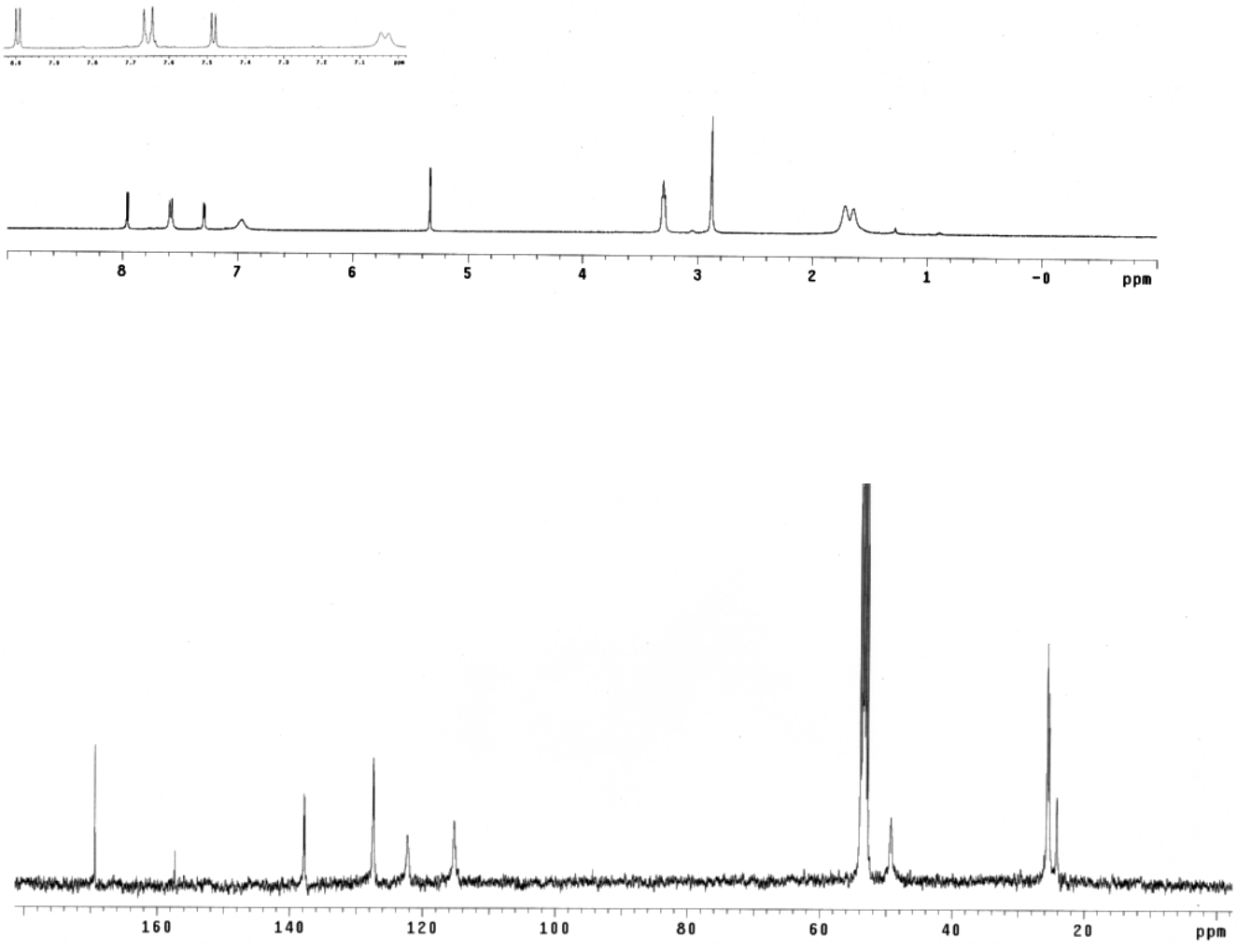
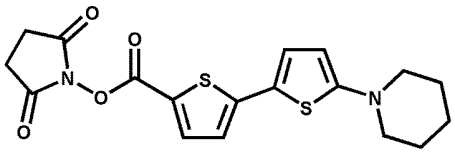


Figure 1S. ^{1}H (CD_2Cl_2 with inset in acetone-d_6) and ^{13}C NMR spectra of compound **1.**



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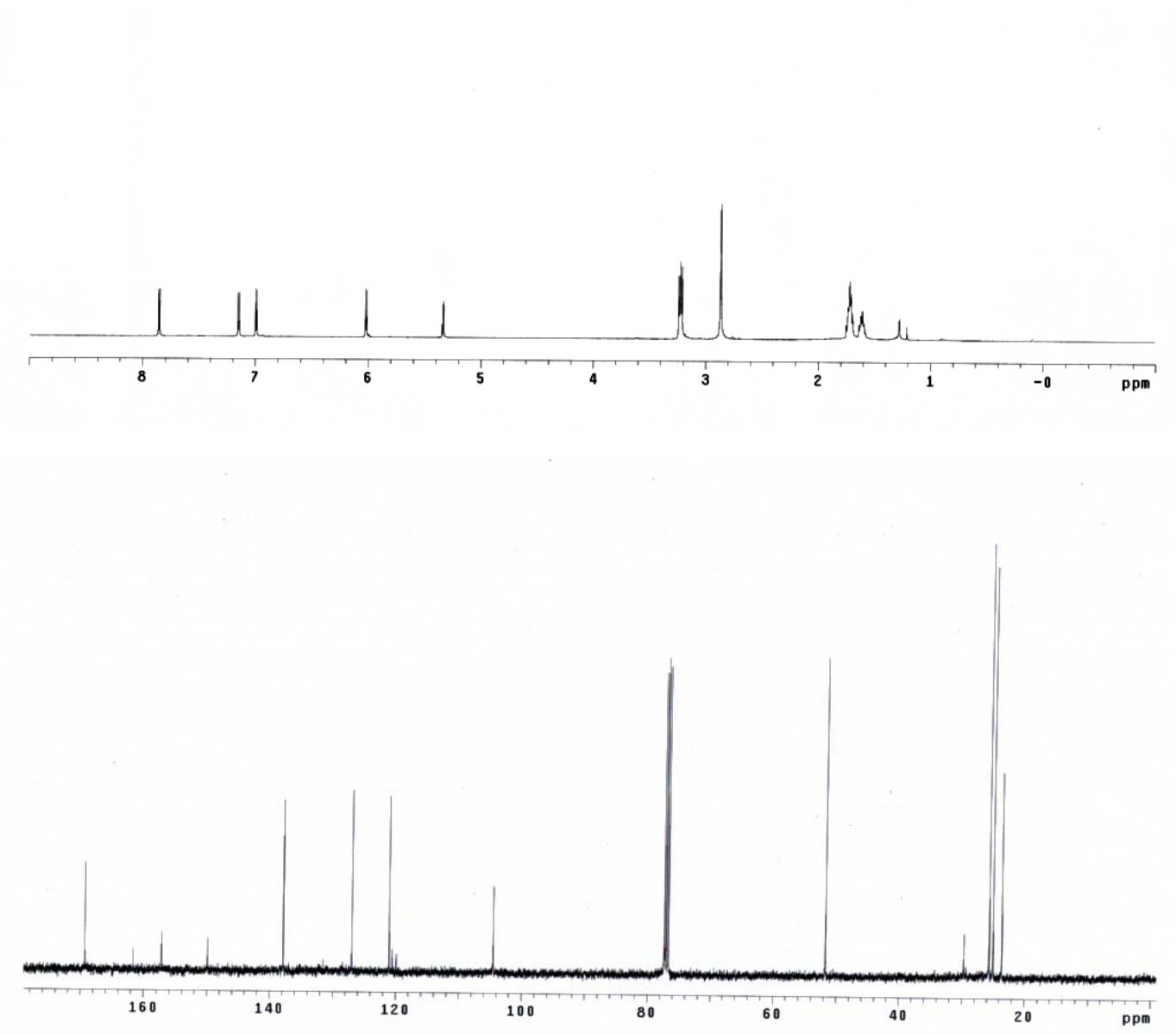
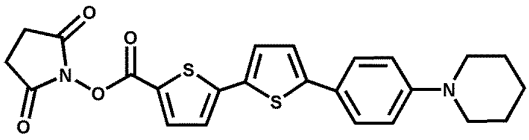


Figure 2S. ^{1}H and ^{13}C NMR spectra of compound **2** in $CDCl_3$**.**



7

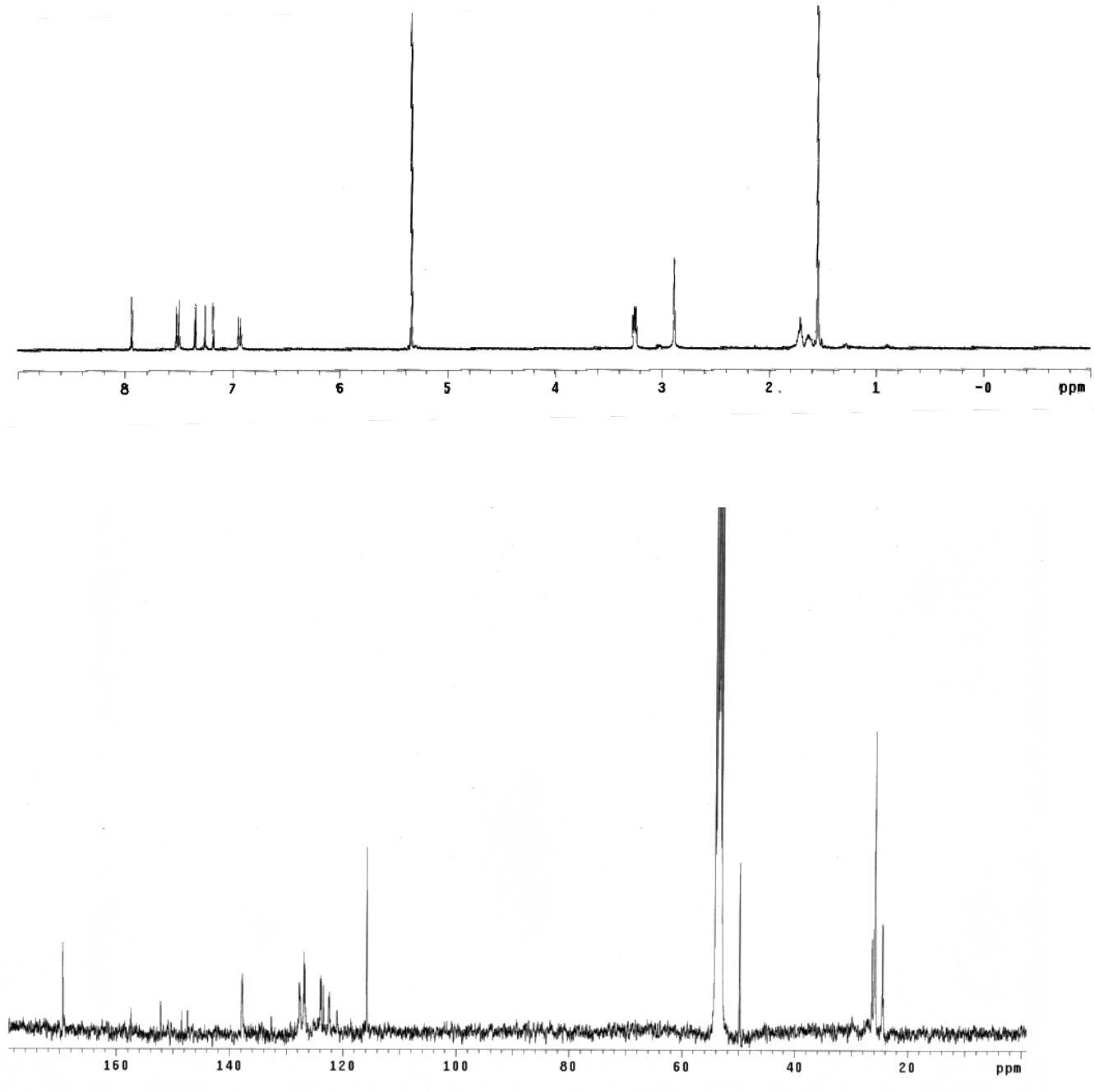
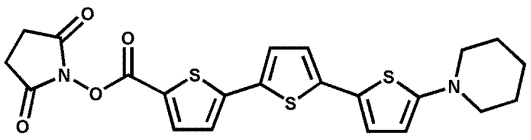


Figure 3S. ^{1}H and ^{13}C NMR spectra of compound **7** in $CDCl_3$**.**



8

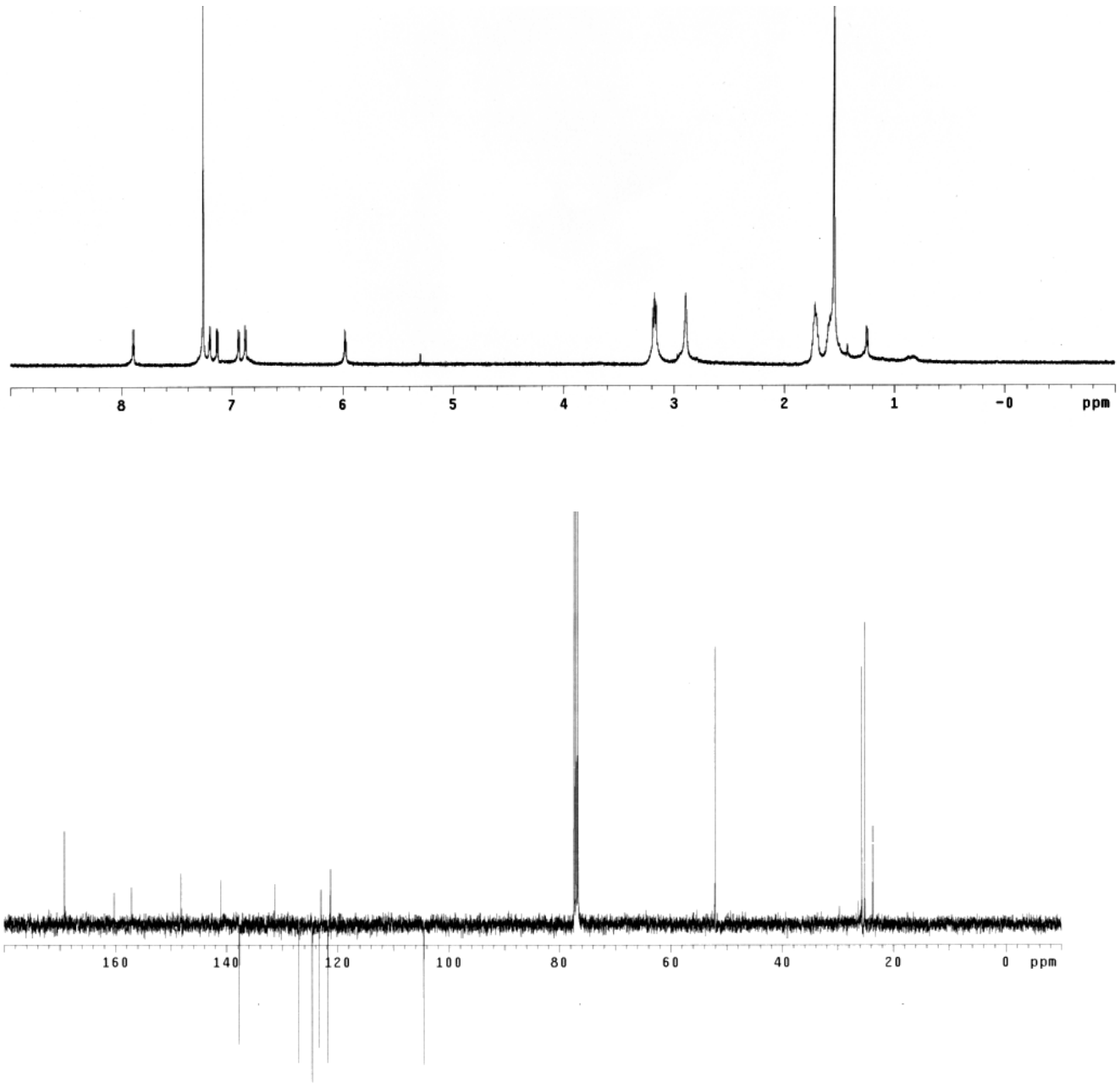
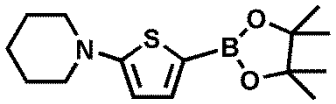
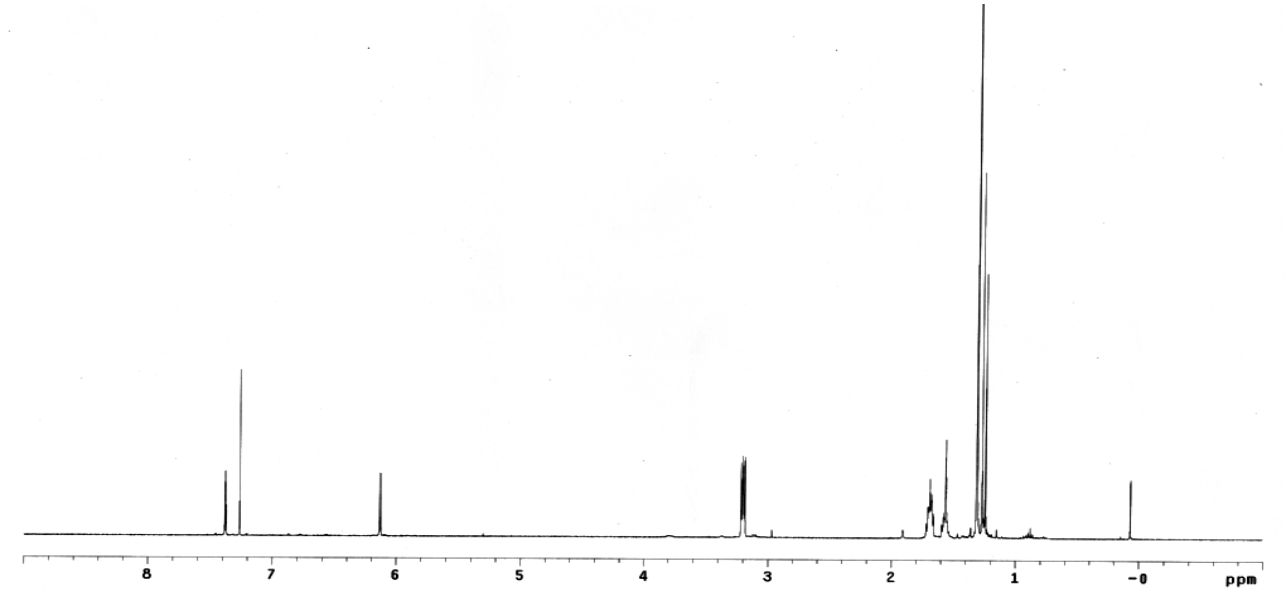
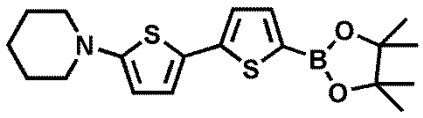


Figure 4S. ^{1}H and ^{13}C NMR spectra of compound **8** in $CDCl_3$.



2b





8b

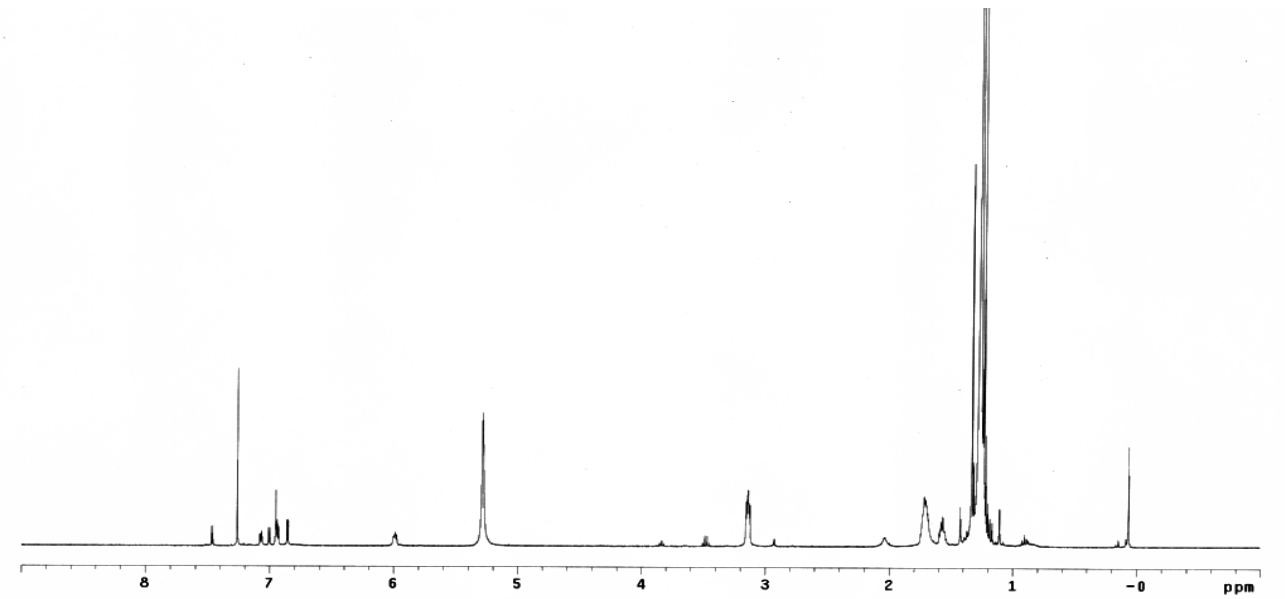
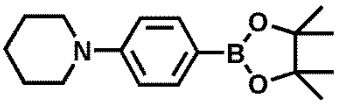


Figure 5S. ^{1}H NMR spectra of compound **2b** and **8d** in $CDCl_3$**.**



1b

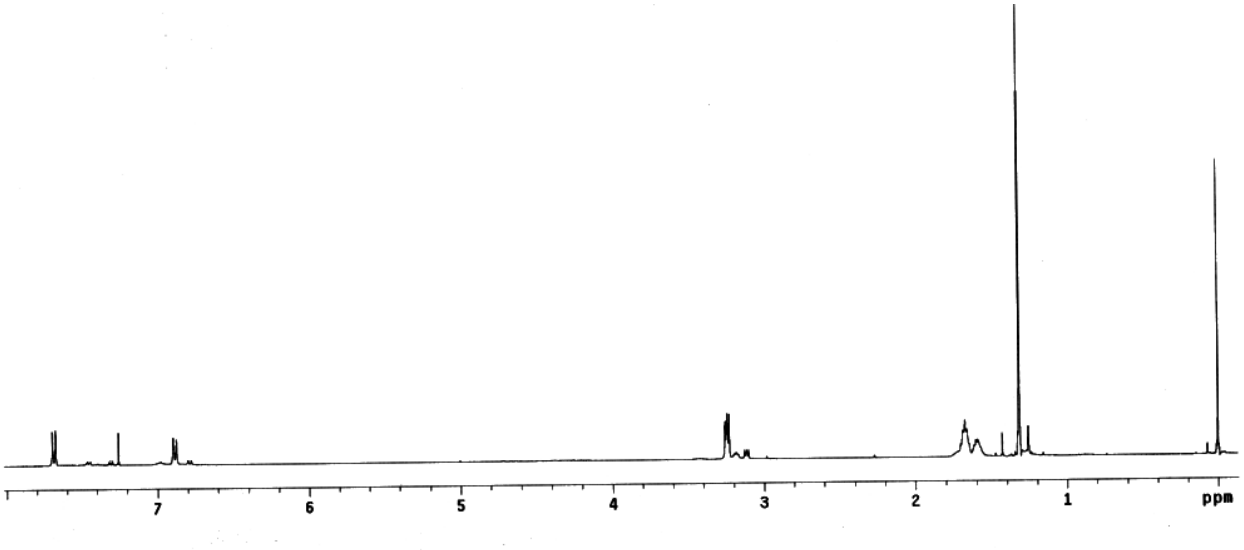


Figure 6S. ^{1}H NMR spectra of compound **1b.**

II. OPTICAL PROPERTIES

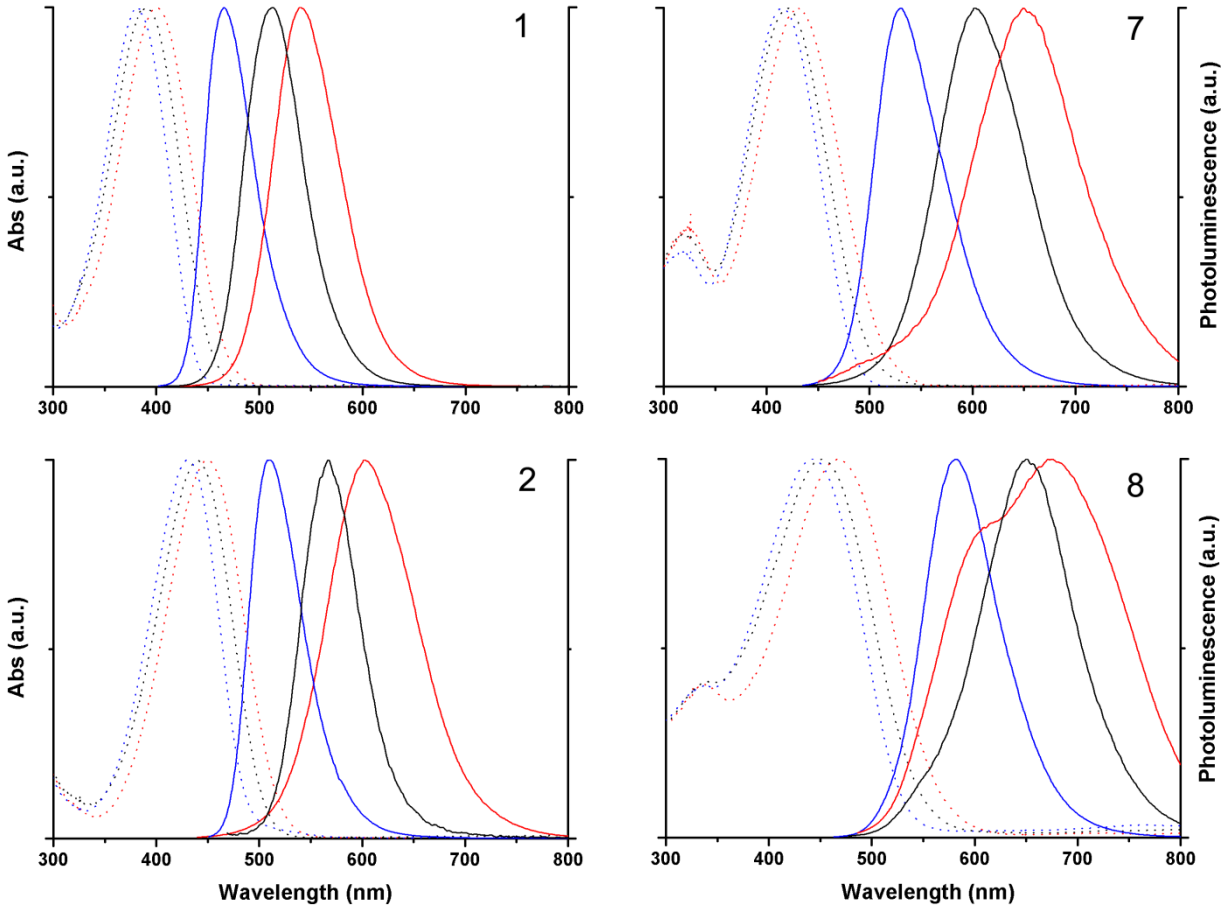


Figure 7S. Absorption and fluorescence spectra of fluorophores **1**, **2**, **7, 8** in toluene (blue), DCM (black) and DMSO (red).

Table 1S. Maximum absorption (λ_{max}, nm) and emission (λ_{PL}, nm) wavelengths and fluorescence lifetimes (τ, ns) of **1-2**,**7-8** in EA and DCM, and fluorescence quantum yield (ϕ) of **7** in DCM.

Item	λ_{max}	λ_{PL}	τ	ϕ^a	λ_{max}	λ_{PL}	τ	ϕ^a
	Ethyl Acetate				Dichloromethane			
1	383	449	2.51	0.70	390	513	2.23	0.62
2	429	533	2.83	0.54	442	548	2.51	0.51
7	412	589	3.00	0.45	421	619	2 .46	0.45
8	448	635	3.24	0.38	458	660	3.39	0.35

[a] Calculated with respect to fluorescein.

Production of reactive singlet oxygen species by fluorophores 1, **2**,**7** and **8**

The typical luminescence of the lowest electronically excited singlet state of molecular oxygen, O_2 ($^1\Delta_g$) with a maximum at ca. 1270 nm was detected upon excitation at 442 nm of air equilibrated toluene solutions of the thiophene fluorophores. The figure below displays the results of the NIR luminescence experiment compared with a standard of 5,10,15,20-tetraphenylporphyrin (TPP) in toluene (ϕ_Δ = 0.70). The measured yield of $^1\Delta_g$ is 0.08, 0.07, 0.05 and 0.08 for **8**, **7**, **2** and **1**, respectively.

The data shown in Figure S11 clearly show that none of the investigated compounds is able to sensitize the production of the reactive singlet oxygen species.

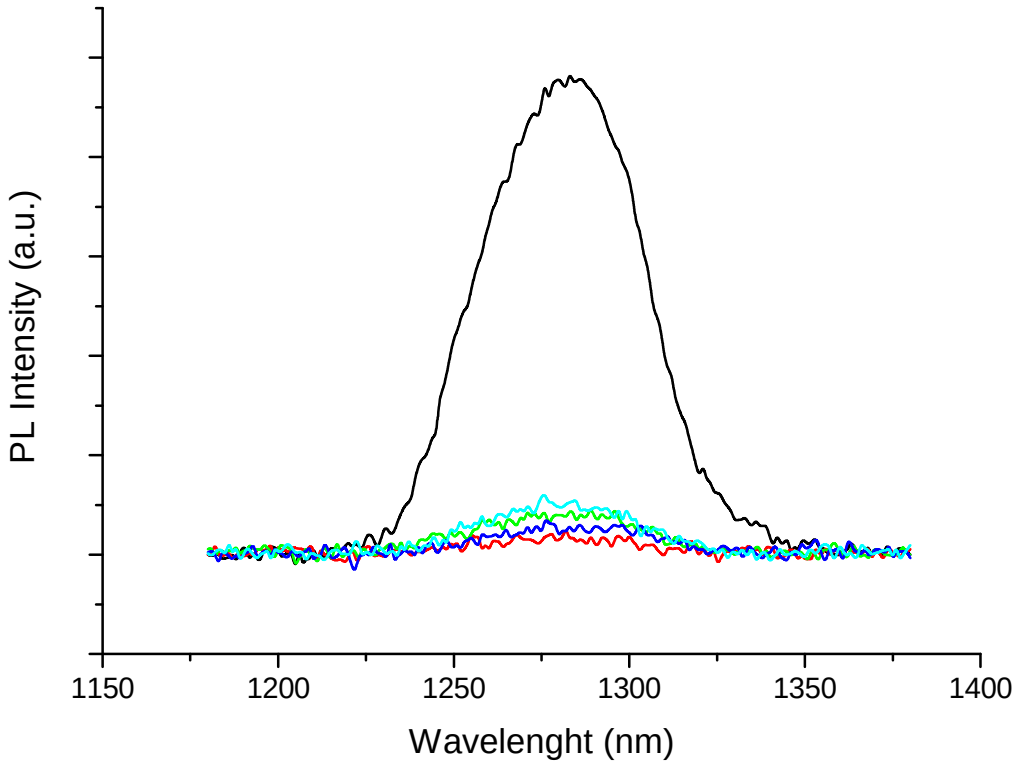


Figure 8S. Sensitised singlet oxygen emission spectra of isoabsorbing TOL solutions of **1** (red), **7** (green), **2** (blue), **8** (magenta) with respect to tetraphenyl porphyrine (black); λ_{exc} = 442 nm.

III. CELL STAINING

General. Tissue culture media and serum were purchased from Sigma, cell line from American Tissue Type Collection (ATTC). The suppliers of the chemicals were: fetal bovine serum (FBS, Sigma, USA), penicillin-streptomycin solution (Sigma, USA), L-glutamine 200 mM (Sigma, USA), DMEM medium (Sigma, USA), sodium pyruvate solution (Sigma, USA), trypsin-EDTA (Sigma, USA), phosphate buffered saline, Dulbecco A (Sigma, USA), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma, USA).

Cell cultures. Mouse embryonic fibroblast cell line (NIH 3T3) was maintained in DMEM supplemented with FBS (10%), penicillin (100 U/mL culture medium), streptomycin (100 µg/mL culture medium), L-glutamine (5%) and sodium pyruvate (5%). Cells were grown in a humidified incubator at 37°C, 5% CO2, and 95% relative humidity.

Live NIH 3T3 cells labeling. Mouse embryonic fibroblasts (NIH 3Laser ScanninT3 cell line) were seeded at a density of 100.000 cells in tissue culture plate in 1 mL of complete culture medium. The fluorophores were dissolved in the minimum amount of DMSO in order to obtain a stock solution and were then administered to cells by adding the appropriate dilution in DMEM serum free to obtain the final concentration of 0.05 $mgmL^{-1}$ and incubated at 37°C in 5% CO2, 95% relative humidity for 1h. At the end of incubation period unbound dye was removed washing the cell cultures with DMEM medium serum free. The samples were examined after 1-24 hours and 7 days by confocal laser scanning microscopy (LSCM). More details in Supporting Information.

Cytotoxicity tests. Mouse embryonic fibroblasts (NIH 3T3) were analyzed with the cytotoxicity test MTT, a reproducible means of measuring the activity of living cells via mitochondrial dehydrogenase activity whose key component is 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide. Mitochondrial dehydrogenases of viable cells cleaved the tetrazolium ring, yielding purple MTT formazan crystals which were insoluble in aqueous solutions. The crystals were dissolved in acidified isopropanol and the resulting purple solution was spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. Dye suspension at 0.05 $mgmL^{-1}$ concentrations were diluted with appropriate cultural medium. The MTT method of cell determination is most useful when cultures are prepared in multiwall plates. NIH 3T3 cells (10^5 cells/mL) were added to well culture plates at 1000 µL/well and incubated at 37°C in 5% CO2, 95% relative humidity for 24-48 and 72 hours with the **1-3** and **7-8** fluorophores suspension. The control was complete culture medium. After an appropriate incubation period, the cultures were removed from the incubator and the MTT solution added in an amount equal to 10% of the culture volume. Then the cultures were returned to incubator and incubated for 3 hours. After the incubation period,

the cultures were removed from the incubator and the resulting MTT formazan crystals were dissolved with acidified isopropanol solution to an equal culture volume. The plates were read within 1 hour after adding acidified isopropanol solution. The absorbance was spectrophotometrically measured at wavelength 570 nm and the background absorbance measured at 690 nm substracted.

The percentage viability was expressed as the relative growth rate (RGR) by equation:

$$RGR = (D_{sample}/ D_{control}) *100\%$$

where D_{sample} and $D_{control}$ are the absorbances of the sample and the negative control. [Mosmann, T.T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* **1983**,12-16]

Isolation of red fluorescent aggregates from NIH 3T3 cell lysate. Fluorescent 'spots' present inside the cytoplasmatic region of the mouse embryonic fibroblast (NIH 3T3) cells after treatment with fluorophore **7**, were purified by whole cell lysates in 50 mM Tris HCl, pH 7.4; 1% Triton X-100; 5 mM EDTA; 150 mM NaCl; 1 mM Na3VO4; 1 mM NaF; 1 mM phenylmethylsulfonyl fluoride (PMSF), in the presence of protease inhibitor cocktail (10 µM benzamidine-HCl and 10 µg each of aprotinin, leupeptin and pepstatin A per mL) followed by incubation at 4°C. **7**-conjugates were left to decant, harvested into fresh reaction tube, washed three times with fresh lysis buffer by centrifugation, and stored at 4°C. Analyses by fluorescence microscopy were performed to confirm the isolation of fluorescent 'spots' from the cells.

SDS-PAGE. Samples dilution of red fluorescent 7-protein conjugates into SDS-loading buffer (1:1) were separated on SDS-polyacrylamide gels without prior heating. Resolved proteins bands were visualized under Coomassie Brillant Blue.

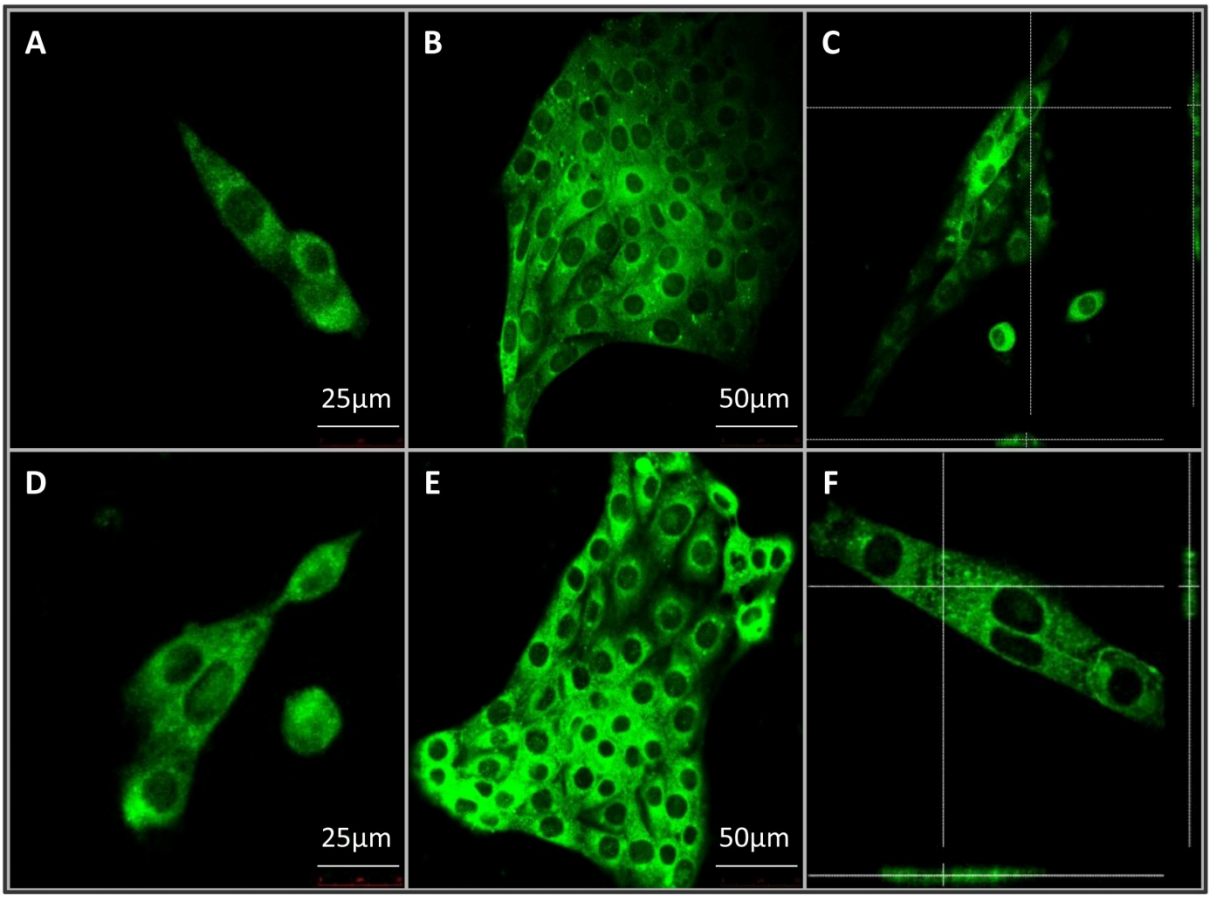


Figure 9S. A), B) LSCM images of live NIH 3T3 cells after 1 hour and 24 hour from treatment with fluorophore **1**. **D, E)** LSCM images of live 3T3 cells after 1 hour and 24 hour from treatment with fluorophore **2**. **C)**, **F)** Cross sections along the z direction (thickness of the slice: 200 nm).

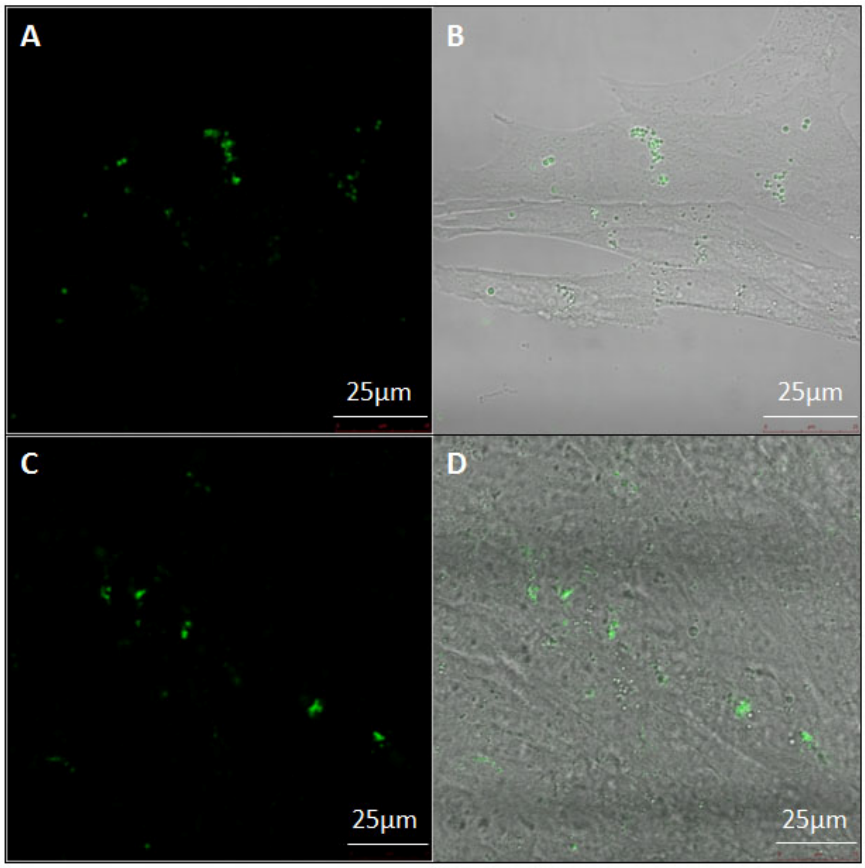


Figure 10S. A), **C)** LSCM images of live NIH 3T3 cells after 192h from treatment with fluorophores **1** and **2**, respectively. **B)**, **D)**, Overlay of light transmission and fluorescence images after 192h from treatment with **1**.

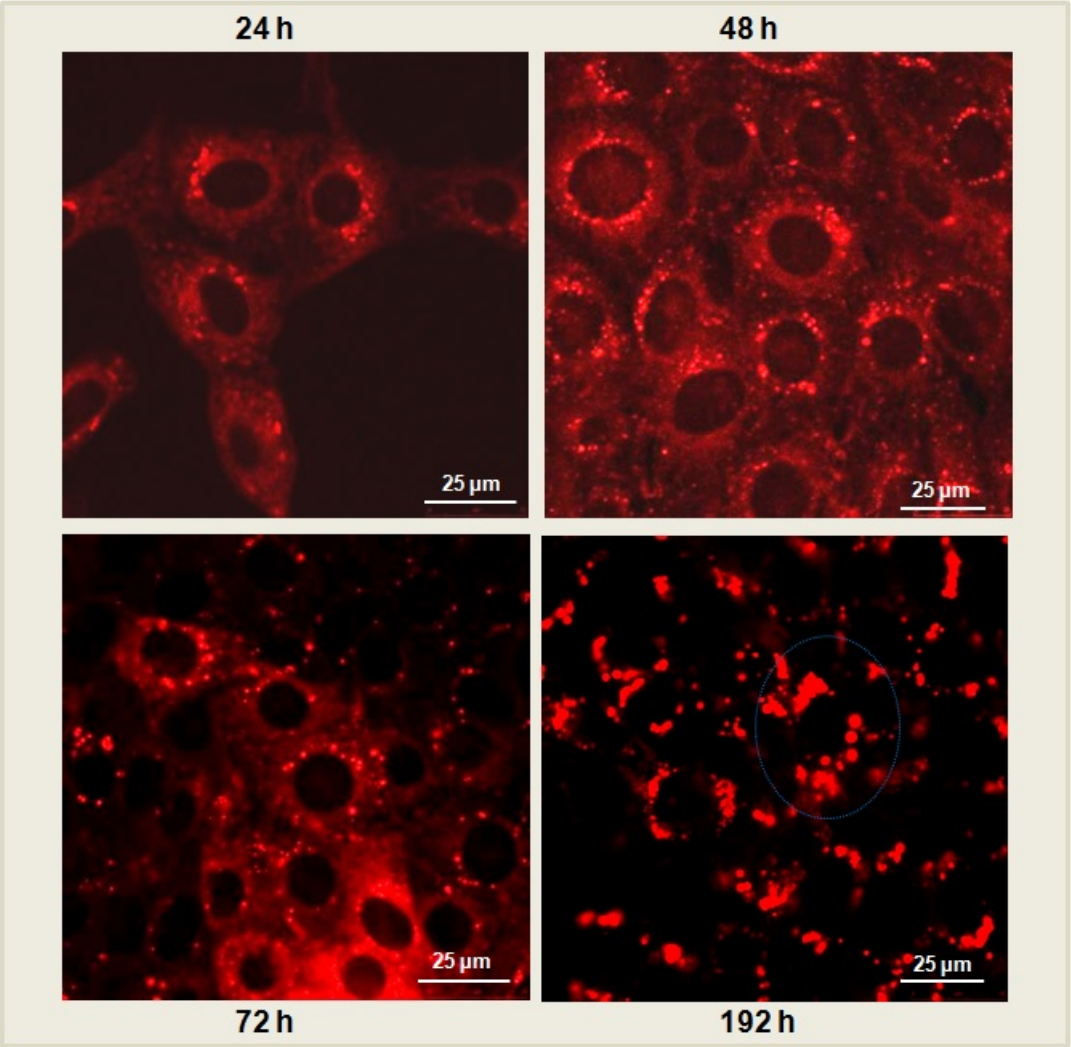


Figure 11S. LSCM images of live NIH 3T3 cells taken 24h, 48h, 72h and 192h after treatment with fluorophore **7**.

Fixed NIH 3T3 cells stained with fluorophores 3 and 8. Mouse embryonic fibroblasts (NIH 3T3 cell line) were seeded at a density of 100.000 cells in tissue culture plate in 1 mL of complete culture medium. Fluorophores **3** and **8** were dissolved in the minimum amount of DMSO in order to obtain a stock solution which was then administered to the cells by adding the appropriate dilution in DMEM serum free to obtain the final concentration of 0.05 $mgmL^{-1}$ and incubated at 37°C in 5% CO_2, 95% relative humidity for 1h. At the end of the incubation period unbound dye was removed

washing the cell cultures with DMEM medium serum free. After 24 hours each sample was rinsed twice with PBS to remove all unattached cells and was fixed in formaldehyde 3.7%. Nuclei were stained with DAPI (Sigma-Aldrich). The samples were examined using a Leica confocal scanning system mounted on a Leica TCS SP5 (Leica Microsystem GmbH, Mannheim, Germany) and equipped with 63X oil immersion objectives and spatial resolution of approximately 200 nm in x-y and 100 nm in z.

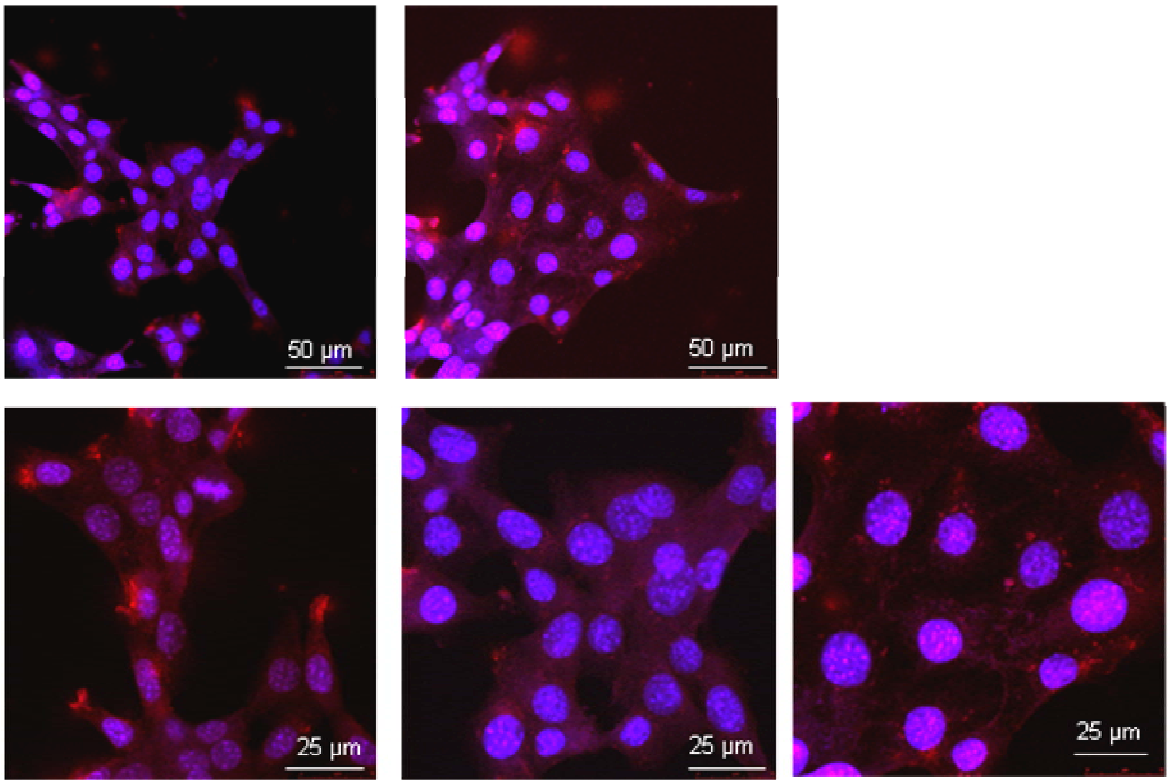


Figure 12S. LSCM images of fixed NIH 3T3 cells incubated with fluorophore **8** (0.05 mg/mL). Green: fluorophore 3. Blue: DAPI.

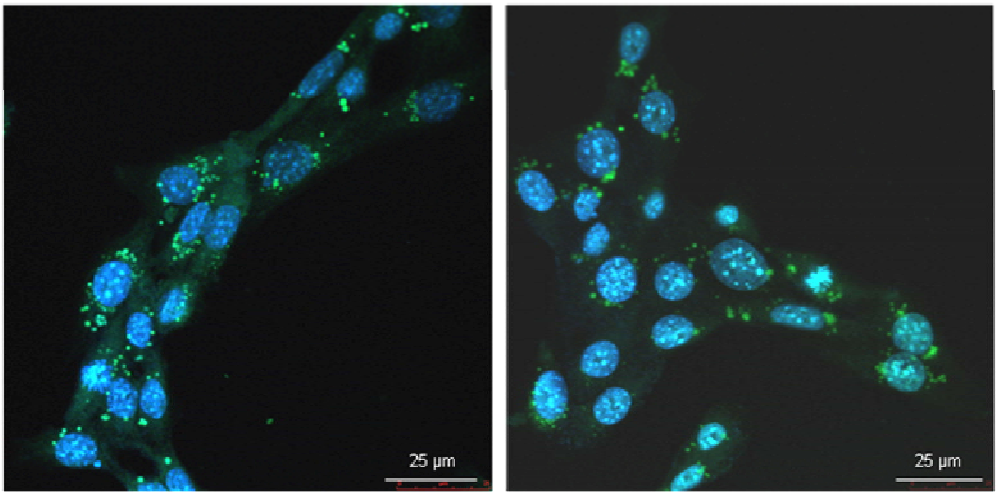


Figure 13S. LSCM images of fixed NIH 3T3 cells incubated with fluorophore **3** (0.05 mg/mL). Red: fluorophore **8**. Blue: DAPI.

IV. THEORETICAL CALCULATIONS

A) To have an idea of the decomposition mechanisms of compounds **1**, **2**,**7** and **8** we carried out theoretical calculations on the photostability of 2-dimethylamino-thiophene, 2-thienyl-carboxylic acid and 2-thiomethyl-thiophene, for comparison.
It has been suggested that the relative population of Dewar and triplet structures has to be considered responsible for the photochemical isomerization or degradation of thiophene derivatives (M. D'Auria *Journal of photochemistry and photobiology A: Chemistry* **2002**, 149, 31-37 and references therein). We have therefore investigated by density functional theory (DFT) calculations the availability of Dewar and triplet structures for 2-dimethylamino-thiophene taken as model for the 2-piperidyl-thiophene moiety of compounds **1**, **2**,**7** and **8**. It was found that the Dewar structure is 15 kcal/mol lower than the triplet one, and therefore it is the Dewar structure that has to be taken into account for the observed behaviour. We have then compared this structure with the Dewar structures of 2-thienyl-carboxylic acid and 2-thiomethyl-thiophene, taken as model for the terminal part of stable oligomers, and of thiophene itself (Figure S12).

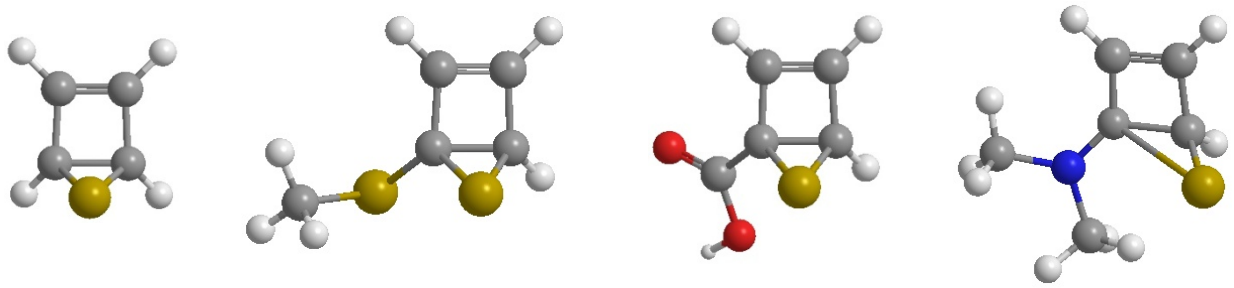


Figure 14S. DFT calculated geometries of the Dewar structures

We note that the presence of a carboxyl group or a thiomethyl group does not perturb the structure of the Dewar thiophene, characterized by the projection of the sulfur atom out of the plane of the carbon atoms and by the equidistance of the sulfur atom from C2 and C5 (1.85 Å). On the contrary, the presence of an ammino group, owing to its mesomeric effect, strongly perturbs the Dewar structure. In fact, the C-N bond is 0.08 Å shorter than the parent compound (1.31 Å *vs* 1.39 Å) and the S-C2 bond is almost broken (2.78 Å). As a consequence, the molecule results more reactive than the more symmetric carboxylic ones. Furthermore, the energy difference between the Dewar and the classic thiophene structure has been calculated to be 57.1 kcal/mol for thiophene itself and 2-thiomethylthiophene, 61.4 kcal/mol for carboxyl derivative and 44.8 kcal/mol for ammine derivative. This latter results therein to be at the same time the more available by energy and the more reactive by structure, and can further evolve on other structures more easily than the carboxylated and thiomethylated ones.

B) Conjugative effect

To have an insight into the efficiency of conjugative effect of thiometyl and amino groups on our push-pull chromophores, DFT calculations have been performed on the two N-succinidimyl esters **a,b** as well as on 2,2'-bithiophene itself (**T-T)** for sake of comparison.

Table 2S. Inter-ring torsion ω(°), inter-ring distance d(Å), HOMO-LUMO energy gap ΔE_{H-L}(eV), dipolar moment μ(Debye), absorption wavelength λ_{max}(nm)

Compound *	**ω(°)**	**d(Å)**	**ΔE_{H-L}(eV)**	**μ(Debye)**	**λ_{max}(nm)****
T-T	158	1.451	4.36	0.27	322
CH_3S-T-T-COONSu **a**	164	1.448	3.72	1.27	372
$(CH_3)_2N$-T-T-COONSu **b**	177	1.438	3.22	5.48	410

* Nsu = Nsuccinimidyl group. **ZINDO/S calculations on optimized DFT geometries

We can note that the presence of the push-pull chromophores leads to a significative increase of the planarity of the bithiophene moiety and to a decreasing of the inter-ring distance. Both these geometrical parameters are a good indicator of the efficiency of a conjugative effect. The ammino group, due to its strong mesomeric effect, is by far more efficient than the thiomethyl group. Moreover, it is also more effective in the decreasing of the energy gap and in the batochromic effect of UV absorption.

C) Computational methods.

All calculations were carried out at the B3LYP/6-31G(d) level within the framework of the Gaussian 03W suite of programs.[1] Unrestricted calculations were used for the Dewar structures. Geometries were fully optimized by standard gradient techniques and the final structures were checked by frequency analysis. Zero point vibrational energy corrections were applied without scaling. The Dewar structures were optimized also at the uMP2/6-31+G(d,p) level, but no difference with DFT structures was found out. UV transitions were calculated by ZINDO/S-C.I. (6 x 6) calculations on DFT geometries using the HyperChem integrated package.[2]

1) Gaussian 03, Revision B05, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Pittsburgh PA, 2003.

2) *HyperChem* rel 7.5 from Hypercube Inc. Waterloo, Ontario, Canada