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PAPER

Fluorescence and two-photon absorption of push–pull aryl(bi)thiophenes: structure–property relationships†‡

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Photophysical and TPA properties of series of push–pull aryl(bi)thiophene chromophores bearing electron-donating (D) and electron-withdrawing (A) end-groups of increasing strength are presented. All compounds show an intense intramolecular charge transfer (ICT) absorption band in the visible region. Increasing the D and/or A strength as well as the length of the conjugated path induces bathochromic and hyperchromic shifts of the absorption band as reported for analogous push–pull polyenes. Yet, in contrast with corresponding push–pull polyenes, a significant increase in fluorescence is observed. In particular, chromophores built from a phenyl–bithienyl conjugated path and bearing strong D and A end-groups were found to combine very large one and two-photon brightness as well as strong emission in the red/NIR region. These molecules hold promise as biphotonic fluorescent probes for bioimaging.

1. Introduction

Among the numerous classes of functional π-conjugated organic materials used as organic semiconductors, oligothiophenes have attracted widespread interest for many years[1] due to their electronic/optical properties, high thermal and chemical stability and their relative ease of synthesis. They have been used for the fabrication of (opto)electronic devices such as organic field-effect transistors (OFETs),[2] organic light emitting diodes (OLEDs),[3] photovoltaic solar cells (OPVs and OSCs),[2a,4] optically pumped lasers[5] and molecular nanowires.[6] They have also been applied in applications such as non-linear optics (NLO)[7] and biology.[8] In particular, the past decade has been marked by a growing interest in the design of NLO chromophores for two photon absorption (TPA) applications. These efforts have been driven by the many applications of the TPA phenomenon in various fields including material science, biology and medicine.[9] Owing to the possibility it provides to use red–NIR excitation (allowing deeper penetration in scattering materials and tissues), and to the quadratic dependency of the TPA process on excitation intensity responsible for an intrinsic three-dimensional spatial resolution, technological applications have been developed such as two-photon excited fluorescence (TPEF) laser-scanning microscopy,[10,11] localised two-photon photodynamic therapy (TP-PDT),[12] localised photorelease of bioactive species,[13] 3D data storage,[14] optical power limiting,[15] and 3D microfabrication.[9a,15f,16] In particular, the increased popularity of TPEF microscopy for biology applications has motivated numerous efforts in the design of optimised fluorophores having large two-photon brightness (*i.e.* $\sigma_2\Phi$ where σ_2 is the TPA cross-section and Φ the fluorescence quantum yield).[9f,11e–h,15e,17] In addition, for easier detection in biological media and tissues, red-emitting fluorophores are particularly helpful. In this context, we have been interested in investigating the potentialities of recently published push–pull oligothiophene derivatives whose second-order optical responses have been shown to be of interest.[7c,f,18] Interestingly, (push–pull) oligothiophene derivatives have been largely described in the literature for organic solar cells purpose[4a,d,19] but, to the best of our knowledge, only scarce examples of oligothiophene derivatives have been investigated for two-photon[20] or three-photon[21] excited photoluminescence up to now.

In this context, we herein report the detailed study of the fluorescence and TPA properties of series of push–pull aryl(bi)thiophene chromophores bearing various electron-donating (OR, NR_2) groups and electron-withdrawing (formyl, dicyanovinyl and barbiturate) end-groups and either one or two thienyl units in the conjugated path. This allowed us to derive structure–property relationships aiming at optimisation of push–pull chromophores for TPEF microscopy.

2. Results and discussion

2.1 Compounds

All derivatives were easily obtained by Knoevenagel condensations starting from 2-formyl-5-arylthiophenes or 5-aryl-5′-

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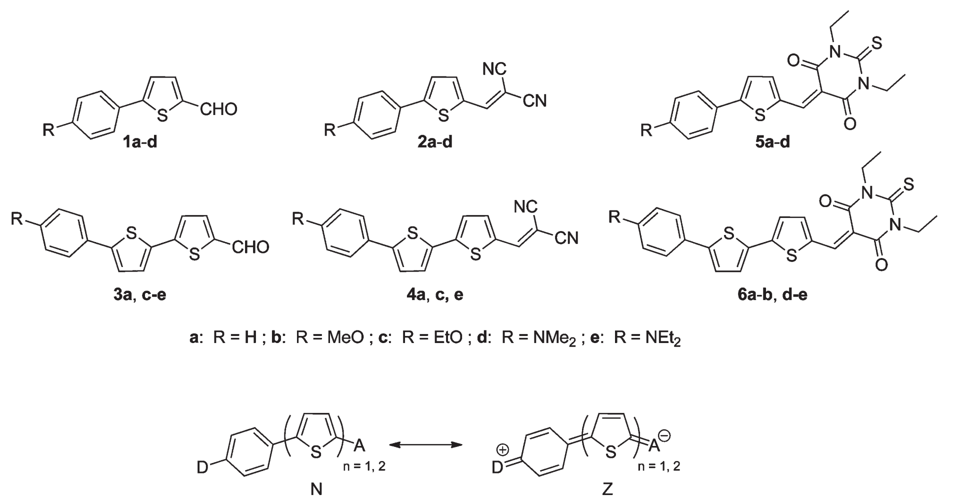
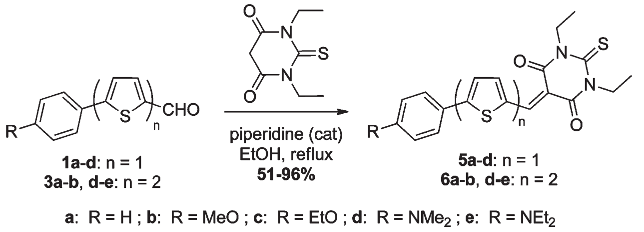


Scheme 1 Structure of compounds **1–6** and the neutral (N) and zwitterionic (Z) limiting-resonance forms.

formyl-2,2′-bithiophenes.[7f,18a] As reported earlier, 2-formyl-5-arylthiophenes are easily obtained *via* the Vilsmeier–Haack–Arnold reaction whereas formyl-substituted 5-aryl-2,2′-bithiophenes are easily obtained *via* the Suzuki coupling of functionalised aryl boronic acids with 5′-bromo-5-formyl-2,2′-bithiophene.[18a]

In the present investigation, different formyl precursors **1a–d** and **3a**, **c–e** bearing different electron-donating end-groups (D) and having arylthiophene (**1**) or arylbithiophene (**3**) π conjugated linkers[7f,18a] were selected in order to evaluate the influence of the electronic strength of the D substituents and of the length of the π-conjugated bridge on the fluorescence and TPA properties of these heteroaromatic aldehydes (Scheme 1). In order to increase the push–pull character of the derivatives, we also shifted to popular strong electron-withdrawing (A) end-groups such as dicyanovinyl (DCV) and 1,3-diethyl-2-thioxodihydropyrimidine-4,6-dione also known as 1,3-diethyl-2-thiobarbiturate (DETB). Such A end-groups have been shown to lead to very large optical non-linearities (both second- and third-order)[7g,22] as well as long distance photo-induced charge transfer[23] in push–pull polyenes having dialkylamino donating end-groups. The dicyanovinyl derivatives **2** and **4** (already reported[7f]) and DETB derivatives (series **5** and **6**) were prepared in a similar way as related push–pull polyenes[7g,22] by reacting aldehyde precursors **1** and **3** with malononitrile or 1,3-diethyl-2-thiobarbituric acid in refluxing ethanol, in the presence of a catalytic amount of piperidine (Scheme 2). New derivatives **5** and **6** were characterised by ^{1}H and ^{13}C NMR, IR, elemental analyses and HRMS.



Scheme 2 Synthesis of thiobarbituric acid derivatives **5** and **6**.

2.2 Photophysical properties

The photophysical properties of derivatives **1–6** are collected in Table 1.

Absorption. The absorption spectra in solution of all derivatives display an intense low-energy absorption band located in the near UV or visible region depending on the strength of the D and A end-groups as well as on the length of the conjugated path. This strong band, associated with high molar extinction coefficients (ranging from 2.2 to 6 × 10^4 mol^{-1} L cm^{-1}), can be ascribed to an intramolecular charge transfer (ICT) transition, as is the case for analogous related push–pull polyenes.[7g,22,23] In the case of the red-shifted chromophores **4e**, **5a–d** and **6a–b**, **d**, **e** having the longer conjugated path and bearing the stronger D/A pairs, a second lower-intensity higher-energy absorption band located in the 350–450 nm range is also observed. This additional band can be ascribed to a higher energy π–π^* transition.

End-groups effect on the ICT absorption band. As previously observed for series **1–4**, comparison of the series of thiobarbituric acid derivatives **5** and **6** shows that increasing the electron-releasing strength of the D end-group (H → OMe → NEt$_2$) induces a marked red-shift of the absorption band (Fig. 1). Similarly increasing the electron-withdrawing strength of the A end-group (CHO → DCV → DETB) in series **1**, **2**, **5** leads to a significant bathochromic and hyperchromic shift as well as marked narrowing of the main absorption band (Fig. 1). The same trend is also observed for the bithienyl series **3**, **4** and **6**. The bathochromic and hyperchromic effects as well as the narrowing of the ICT band induced by increasing either D or A strength indicate that the polarisation increases in the ground state (corresponding to an increase of the contribution of the zwitterionic mesomeric form (Z) in the description of the electronic distribution) shifting the electronic structure towards a more "cyanine-like" distribution (Scheme 1).[24] This behaviour is similar to that observed for related push–pull polyenes (*i.e.* having NR$_2$ donating end-groups and DCV or TETB acceptor end-groups).[7g,22]

Table 1 Photophysical properties of compounds **1–6** in chloroform solution

	D	A	π	λ_{abs}^{max} (nm)	Log ε^{max} (M^{-1} cm^{-1})	FWHM (10^3 cm^{-1})	λ_{em}^{max} (nm)	Stokes shift (10^3 cm^{-1})	Φ_f (%)	τ (ns)	k_r (10^8 s^{-1})	k_{nr} (10^9 s^{-1})
1a	H	CHO	Ph–Thien	331	4.35	4.6	375	3.6	0.4	—	—	—
1b	OMe	CHO	Ph–Thien	352	4.34	4.6	427	5.0	13.7	0.6	2.3	1.41
1d	NMe_2	CHO	Ph–Thien	408	4.45	4.3	507	4.8	84.5	2.9	2.9	0.06
2a	H	$CH{=}C(CN)_2$	Ph–Thien	404	4.54	3.8	443	2.2	0.04	—	—	—
2b	OMe	$CH{=}C(CN)_2$	Ph–Thien	431	4.56	3.9	502	3.3	0.2	—	—	—
2c	OEt	$CH{=}C(CN)_2$	Ph–Thien	433.5	4.54	3.9	502	3.2	0.2	—	—	—
2d	NMe_2	$CH{=}C(CN)_2$	Ph–Thien	511.5	4.55	3.5	609	3.1	13.5	0.6	2.1	1.37
3a	H	CHO	$Ph–(Thien)_2$	390.5	4.39	4.4	469	4.3	35	1.2	3.0	0.55
3c	OEt	CHO	$Ph–(Thien)_2$	403	4.38	4.5	505	5.0	79	2.5	3.2	0.09
3d	NMe_2	CHO	$Ph–(Thien)_2$	437.5	4.45	4.8	601	6.2	77	3.4	2.3	0.07
3e	NEt_2	CHO	$Ph–(Thien)_2$	448.5	4.40	4.6	612	6.0	74	3.4	2.2	0.08
4a	H	$CH{=}C(CN)_2$	$Ph–(Thien)_2$	468	4.59	3.8	546	3.0	0.5	—	—	—
4c	OEt	$CH{=}C(CN)_2$	$Ph–(Thien)_2$	486.5	4.61	3.9	599	3.9	5	0.4	1.4	2.64
4e	NEt_2	$CH{=}C(CN)_2$	$Ph–(Thien)_2$	551.5	4.59	4.0	727	4.4	34	2.7	1.2	0.24
5a	H	DETB	Ph–Thien	464.5	4.71	3.2	524	2.4	0.04	—	—	—
5b	OMe	DETB	Ph–Thien	491.5	4.74	3.2	555	2.3	0.1	—	—	—
5c	OEt	DETB	Ph–Thien	494	4.72	3.2	561	2.4	0.2	—	—	—
5d	NMe_2	DETB	Ph–Thien	571.5	4.79	2.9	669	2.6	8.7	0.4	0.22	2.28
6a	H	DETB	$Ph–(Thien)_2$	526.5	4.72	3.2	596	2.2	0.5	—	—	—
6b	OMe	DETB	$Ph–(Thien)_2$	541	4.74	3.3	644	3.0	8.8	0.2	0.44	4.56
6d	NMe_2	DETB	$Ph–(Thien)_2$	587.5	4.66	3.8	781	4.2	5.6	1.1	0.05	0.84

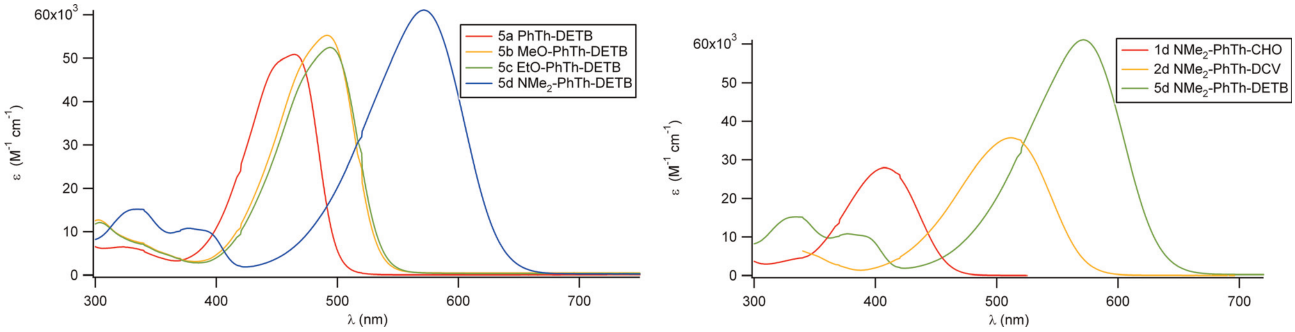


Fig. 1 UV-vis absorption spectra of compounds **5a–d** and **1,2,5d**: end-groups effect (donor strength: left and acceptor strength: right).

Length and connector effect. As expected, increasing the length of the π-conjugated bridge also has a marked influence on the photophysical properties. As an illustration, comparison of related derivatives **5** and **6** bearing strong DETB emphasises that increasing the number of thienyl moieties induces a marked bathochromic shift of the absorption band (Fig. 2), as already reported for push–pull derivatives bearing formyl or DCV acceptors (series **1**, **3** and **2**, **4** respectively). This bathochromic shift is accompanied by a significant broadening in relation to a reduced contribution of the zwitterionic mesomeric form due to the higher cost of charge separation in bithienyl derivatives (Scheme 1). Such a trend was also observed for related push–pull polyenes.[7g,22] Furthermore, comparison of the absorption characteristics of push–pull (oligo)thienyl compounds **1d**, **2d**, **5d** bearing NMe_2 electron-releasing substituents and those of their polyenic analogues indicates that replacing two double bonds by one thienyl unit in the conjugated path leads to a clear blue-shift of the absorption maximum. Replacement of four double bonds by two thienyl units has a similar effect as indicated by comparison of absorption data of compounds **3e**, **4e**, **6d** and their polyenic analogues. This hypsochromic effect indicates a lower delocalisation in oligothienyl push–pull derivatives (*i.e.* lower contribution of the Z form as compared to analogous polyenes having the same number of double bonds in the conjugated path) in relation to the cost of charge separation associated with the loss of aromaticity of the thienyl units (Scheme 1). We observe that the largest effects are obtained for derivatives having

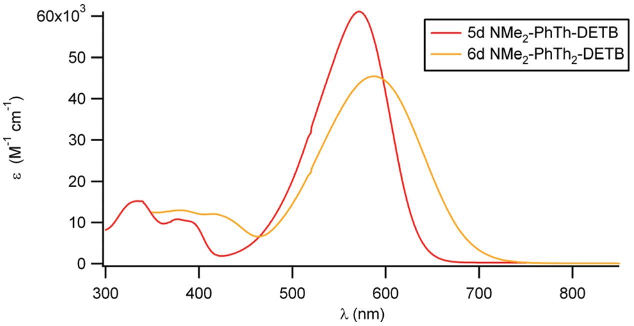


Fig. 2 UV-vis absorption spectra of push–pull compounds **5–6,d**: effect of π-connector length.

aldehydes and DETB (*i.e.* 1415 cm^{-1} for compound **1d**, 1290 cm^{-1} for compound **3e**, 1900 cm^{-1} for compound **5d** and 1850 cm^{-1} for compound **6d**).

Fluorescence. Interestingly, in contrast to analogous push–pull polyenes,[23b,d] a number of derivatives from series **1–6,a–d** show significant fluorescence. This can be ascribed to the lower conformational stability of the (bi)thienyl π-linker as compared to the polyenic chain, resulting in repression of efficient vibrational deactivation processes.

As observed from Table 1, the fluorescence characteristics are strongly dependent on the nature of the end-groups, as well as on the length of the conjugated path.

End-groups effect. As was observed for absorption, increasing either the electron-releasing or electron-withdrawing character of the end-groups induces a marked bathochromic shift of the emission band (Fig. 3), in agreement with the ICT nature of the transition. Moreover the fluorescence quantum yield tends to be higher for stronger donors. Only push–pull aldehydes **3** bent this rule since they display high fluorescence quantum yield values (up to 79%) regardless of the nature of the D end-group. As a result, all derivatives having strong D end-groups (*i.e.* **d,e** derivatives, D = NR_2) show noticeable fluorescence, indicative of the π–π* nature of the lowest energy excited state due to the ICT character of the transition which shifts its energy below that of the n–π* transition.

In contrast, considering both the thienyl **1**, **2**, **5** and bithienyl **3**, **4**, **6** series and comparing data for compounds having the same D substituent but different A end-groups (1 → 2 → 5 or 3 → 4 → 6), we observe that increasing the A strength (CHO → DCV → DETB) induces both a marked decrease of the Stokes shift and a drastic decrease of the fluorescence quantum yields (Table 1). The decrease of the Stokes shift values parallels the narrowing of the ICT absorption band and emission and absorption red shifts and can be related to the shift of the electronic structure towards the cyanine limit. The steady decrease of the fluorescence quantum yield can be ascribed to the combined effect of the reduction of the radiative decay rate (in connection with the red-shifted emission)[25] and the increase of the non-radiative decay rates (see Table 1). In some cases (**2d**, **4c**, **5d**, **6b**), the large non-radiative decay rates suggest the possible onset of competing processes occurring in the excited state such as photoinduced electron transfer.

Length effect. Interestingly, the emission is significantly red-shifted when the conjugated path is lengthened (phenyl–thienyl → phenyl–bithienyl) as illustrated in Fig. 4 and indicated by comparison of the corresponding chromophores of series **3**, **4**, **6** and **1**, **2**, **5**. This red-shift is even more pronounced than that for absorption resulting in very large Stokes shift values (Table 1) and red or even NIR emission for bithienyl derivatives combining good donor (OR, NR_2) and acceptor (DCV, DETB) end-groups. Even more strikingly, the increased length induces a marked fluorescence enhancement: whatever the series, bithienyl

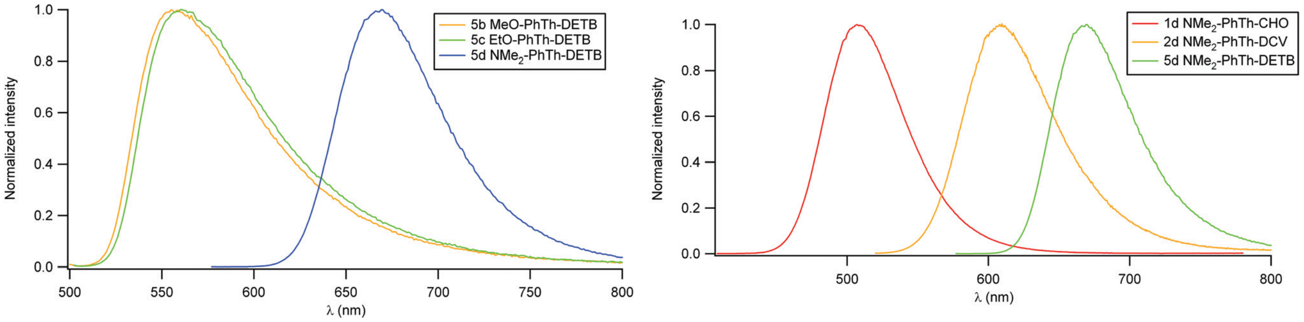


Fig. 3 Fluorescence emission spectra of push–pull compounds **5b–d** and **1,2,5d**: end-groups effect (donor strength: left and acceptor strength: right).

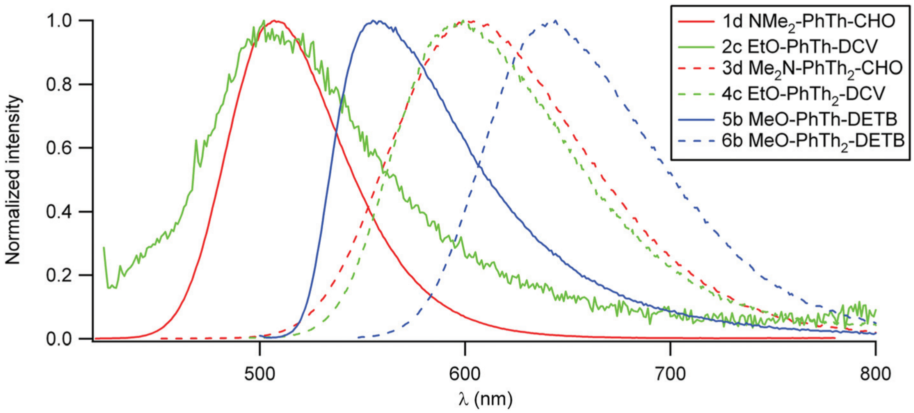


Fig. 4 Fluorescence emission spectra of push–pull compounds **1,3d**; **2,4c** and **5–6c**: length effect.

derivatives are found to be more fluorescent than their thienyl analogues (except for the case of compound **6d** whose low radiative decay rate is responsible for a slight loss of fluorescence). Hence, the bithienyl aldehyde **2a** displays an intense fluorescence (fluorescence quantum yield value of 35%) even in the absence of an electron-releasing *para*-substituent on the aromatic ring, thanks to the combined donor and transmitter nature of the thiophene moiety.

As a result of combined end-groups and length effects, push–pull derivatives built from the bithienyl π-connector and bearing good donor/acceptor end-groups (OMe, NR_2/DCV, DETB: **4e**, **6b,d**) are found to be good red to NIR emitters.

2.3 Two-photon absorption

Based on their fluorescence, we were able to determine the TPA characteristics of the derivatives **1b**, **1d**, **2b–d**, **3**, **4c**, **4e**, **5d** and **6d–e** by investigating their two-photon induced fluorescence in solution. TPA spectra were obtained in the 700–1200 nm spectral range through femtosecond two-photon excited fluorescence experiments and by following the methodology described by Webb and collaborators.[26] The corresponding data are gathered in Table 2. All compounds show a broad TPA band[27] (Fig. 5) whose maximum is located at about twice the wavelength of the one-photon absorption (OPA) band, which indicates that the lowest excited state (ICT transition) is both one and two-photon allowed as expected for push–pull derivatives. In addition, fluorescent derivatives **4e** and **5–6d** having strong D and A groups show *an additional TPA band at higher energy* corresponding to the less intense absorption band located in the near UV (**4e**, **5d**) or violet (**6d**) region. Interestingly, whereas the ICT band is both one- and two-photon allowed, the second absorption band corresponding to a higher excited state is much more strongly two-photon allowed, leading to high TPA cross-section values in a wide spectral range of interest for biological imaging (typically 1250 GM at 800 nm and 1570 GM at 1170 nm for the red-emitting compound **5d**). Such a phenomenon has already been observed in octupolar merocyanines.[28]

End-groups effect. For the different series, comparisons of chromophores bearing different electron-releasing groups showed that increasing the donor strength leads both to a bathochromic shift of the TPA band (in connection with the ICT band red-shift) and a marked enhancement of the maximum TPA cross section (σ_2) values (Table 2) as illustrated in Fig. 5. A similar trend is obtained when increasing the acceptor strength (Fig. 5). Interestingly, this hyperchromic effect is much more

Table 2 TPA properties of compounds **1–6** in chloroform solution

	D	A	π	$2\lambda_{ICT}$ (nm)	λ_{TPA}^{max1} (nm)	σ_2 (GM) at λ_{TPA}^{max1}	λ_{TPA}^{max2} (nm)	σ_2 (GM) at λ_{TPA}^{max2}
1b	OMe	CHO	Ph–Thien	704	730	56	—	—
1d	NMe_2	CHO	Ph–Thien	816	820	124	—	—
2a	H	$CH{=}C(CN)_2$	Ph–Thien	808	820	28	—	—
2b	OMe	$CH{=}C(CN)_2$	Ph–Thien	862	880	95	—	—
2c	OEt	$CH{=}C(CN)_2$	Ph–Thien	867	880	108	—	—
2d	NMe_2	$CH{=}C(CN)_2$	Ph–Thien	1023	1050	176	—	—
3a	H	CHO	Ph–$(Thien)_2$	781	770	47	—	—
3c	OEt	CHO	Ph–$(Thien)_2$	806	820	115	—	—
3d	NMe_2	CHO	Ph–$(Thien)_2$	875	890	208	—	—
3e	NEt_2	CHO	Ph–$(Thien)_2$	897	890	253	—	—
4c	OEt	$CH{=}C(CN)_2$	Ph–$(Thien)_2$	973	930	210	—	—
					1040	125	—	—
4e	NEt_2	$CH{=}C(CN)_2$	Ph–$(Thien)_2$	1103	1100	570	790	780
					1190	600	—	—
5d	NMe_2	DETB	Ph–Thien	1143	1120	428	800	1240
6d[a]	NMe_2	DETB	Ph–$(Thien)_2$	1175	>1170	>1600	—	—
					1100	1180	—	—

[a] Not determined due to excitation and fluorescence wavelengths overlap.

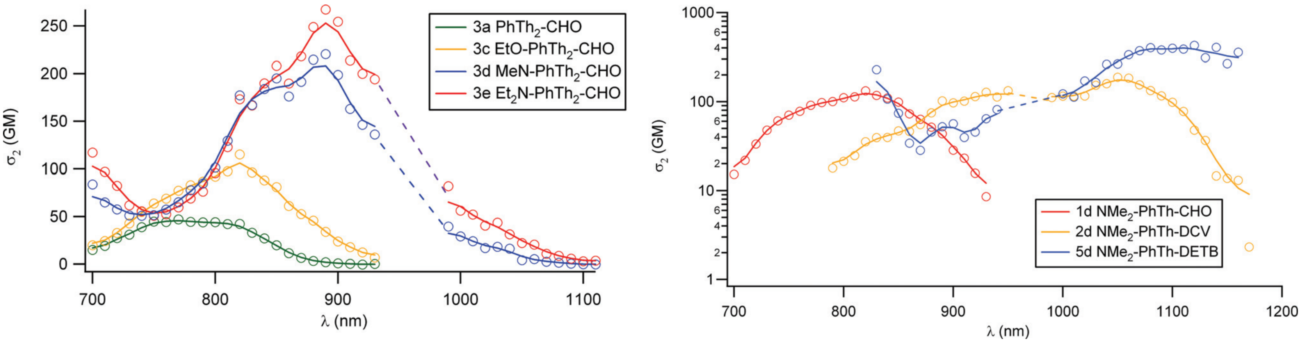


Fig. 5 Two-photon absorption spectra of compounds **3a–e** and **1,2,5d**: end-groups effect (donor strength: left and acceptor strength: right).

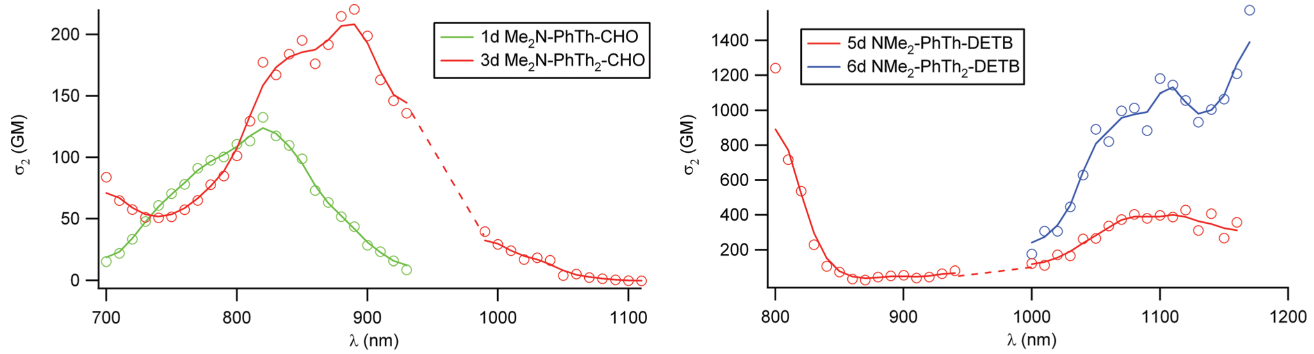


Fig. 6 Two-photon absorption spectra of compounds**1,3d** and **5–6d**: length effect.

pronounced for TPA than OPA in relation to the pronounced ICT character of the transition.[24b]

Length effect. Finally the nature of the π-conjugated bridge strongly influences the TPA properties of the compounds. Increasing the number of thiophene moieties also induces a red-shift of the TPA (paralleling the red-shift of the ICT absorption band) and leads to a marked enhancement of the TPA cross-section values (σ_2) as illustrated in Fig. 6. As a result, push–pull derivatives having two thienyl units and combining strong D and A end-groups (typically D = NR_2 and A = DCV or DETB) were found to show very large maximum TPA cross-sections in the spectral region of interest for bioimaging (1600 GM at 1200 nm for NIR emitting compound **6d**).

3. Experimental section

3.1 Compounds

The synthesis and characterisation of compounds **1–4,a–e** have been described earlier.[7f,18a] New compounds **5–6,a–e** have been synthesised following the same procedure.

Thin layer chromatography was carried out on 0.25 mm thick precoated silica plates (Merck FertigplattenKieselgel 60F$_{254}$). Melting points were measured on a Gallenkamp melting point apparatus. NMR spectra were obtained on a Varian Unity Plus Spectrometer at an operating frequency of 300 MHz for ^{1}H NMR and 75.4 MHz for ^{13}C NMR or a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H NMR and 100.6 MHz for ^{13}C NMR using the solvent peak as an internal reference at 25 °C. All chemical shifts are given in ppm using $\delta_H Me_4Si$ = 0 ppm as a reference and J values are given in Hz. Assignments were made by comparison of chemical shifts, peak multiplicities and J values and were supported by spin decoupling-double resonance and bidimensional heteronuclear HMBC and HMQC correlation techniques. IR spectra were run on an FTIR Perkin-Elmer 1600 spectrophotometer in nujol or in KBr. Elemental analyses were carried out on a Leco CHNS 932 instrument. Low and high resolution mass spectrometry analyses were performed at the "C.A.C.T.I. – Unidad de Espectrometria de Masas", at the University of Vigo, Spain. 1,3-Diethyl-2-thiobarbituric acid was purchased from Sigma-Aldrich and used without further purification.

General procedure for the synthesis of arylthiophene and arylbithiophenethiobarbituric acid derivatives 5–6 from the corresponding formyl precursors 1 and 3 by Knoevenagel condensation. To a solution of 1,3-diethyl-2-thiobarbituric acid (80 mg, 0.38 mmol, 1.2 equiv.) and aldehydes **1** or **3** (0.32 mmol, 1.0 equiv.) in ethanol (20 ml) was added piperidine (1 drop). The solution was stirred at reflux during different reaction times (3–4 h) and cooled until room temperature. The mixture was filtered and the crude solid was washed with diethyl ether. Recrystallisation from dichloromethane–petroleum ether gave the pure compounds.

1,3-Diethyl-dihydro-5-((5′-phenylthiophen-2′-yl)methylene)-2-thioxopyrimidine-4,6(1*H*,5*H*)-dione 5a. Orange solid (51%). Mp 223–224 °C. IR(KBr) ν 3088, 1657, 1547, 1506, 1394, 1331 cm^{-1}. ^{1}H NMR (DMSO-d$_6$) δ 1.20 (m, 6 H, 2 × NCH_2CH_3), 4.44 (m, 4 H, 2 × NCH_2CH_3), 7.50 (m, 3 H, 3″-H, 4″-H and 5″-H), 7.90 (m, 3 H, 2″-H, 6″-H and 4′-H), 8.31 (d, 1 H, J = 4.2 Hz, 3′-H), 8.66 (s, 1 H,$=CH$).^{13}C NMR (CDCl$_3$) δ: 12.4, 12.5, 43.2, 44.0, 110.4, 124.8, 126.8, 129.2, 130.0, 136.8, 147.2, 149.8, 152.1, 159.8, 161.0, 161.4, 178.7. Anal. Calcd for $C_{19}H_{18}N_2O_2S_2$: C, 61.60; H, 4.90; N, 7.56; S, 17.3. Found: C, 61.70; H, 5.04; N, 7.58; S, 16.9.

1,3-Diethyl-5-((5′-(4″-methoxyphenylthiophen-2′-yl)-methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione 5b. Red solid (73%). Mp 221–223 °C. IR(KBr) ν 3089, 2948, 1656, 1603, 1493, 1395, 1333, 1257, 1031 cm^{-1}. ^{1}H NMR (DMSO-d$_6$) δ 1.20 (m, 6 H, 2 × NCH_2CH_3), 3.83 (s, 3 H, OCH_3), 4.44 (m, 4 H, 2 × NCH_2CH_3), 7.06 (d, 2 H, J = 8.9 Hz, 3″-H and 5″-H), 7.79 (d, 1 H, J = 4.2 Hz, 4′-H), 7.86 (d, 2 H, J = 8.9 Hz, 2″-H and 6″-H), 8.28 (d, 1 H, J = 4.2 Hz, 3′-H), 8.64 (s, 1 H, $C=CH$). ^{13}C NMR (DMSO-d$_6$) δ 12.4, 12.5, 43.1, 43.9, 55.5, 109.5, 114.7, 123.9, 125.8, 128.4, 135.9, 147.7, 149.7, 159.9, 161.1, 161.4, 162.1, 178.7. Anal. Calcd for $C_{20}H_{20}N_2O_3S_3$: C, 59.98; H, 5.03; N, 6.99; S, 16.0. Found: C, 59.75; H, 5.08; N, 7.00; S, 15.7.

1,3-Diethyl-5-((5′-(4″-ethoxyphenylthiophen-2′-yl)-methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione 5c. Violet solid (58%). Mp 198–199 °C. IR(KBr) ν 3081, 2934, 1651, 1603, 1486, 1388, 1331, 1255, 1052 cm^{-1}. ^{1}H NMR (DMSO-d$_6$) δ 1.20 (m, 6 H, 2 × NCH_2CH_3), 1.35 (t, 3 H, J = 6.9 Hz, OCH_2CH_3), 4.10 (q, 2 H, J = 6.9 Hz, OCH_2CH_3), 4.44 (m, 4 H, 2 × NCH_2CH_3), 7.05 (d, 2 H, J = 8.6 Hz, 3″-H and 5″-H), 7.79

(d, 1 H, *J* = 4.3 Hz, 4′-H), 7.86 (d, 2 H, *J* = 8.6 Hz, 2″-H and 6″-H), 8.29 (d, 1 H, *J* = 4.3 Hz, 3′-H), 8.64 (s, 1 H, C=C*H*). ^{13}C NMR (DMSO-d$_6$) δ 12.5, 14.7, 43.1, 43.9, 63.7, 109.4, 115.1, 123.9, 125.6, 128.4, 135.9, 147.8, 149.7, 159.9, 160.8, 161.1, 162.3, 178.7. MS (microTOF) *m*/*z* (%): 415 (M$^+$, 100), 338 (36). HRMS: *m*/*z* (microTOF) for $C_{21}H_{23}N_2O_3S_2$; calcd 415.1145; found: 415.1127. Anal. Calcd for $C_{21}H_{22}N_2O_3S_3$: C, 60.84; H, 5.35; N, 6.76; S, 15.5. Found: C, 60.91; H, 5.30; N, 6.76; S, 15.3.

1,3-Diethyl-5-((5′-(4″-*N*,*N*-dimethylaminophenylthiophen-2′-yl)-methylene)-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione 5d. Violet solid (81%). Mp 260–262 °C. IR(KBr) 3073, 2931, 1649, 1604, 1488, 1387, 1362, 1329 cm^{-1}. ^{1}H NMR (CDCl$_3$) δ 1.35 (m, 6 H, 2 × NCH$_2$C*H*$_3$), 3.11 (s, 6 H, N(C*H*$_3$)$_2$), 4.60 (m, 4 H, 2 × NC*H*$_2$CH$_3$), 6.70 (d, 2 H, *J* = 9.1 Hz, 3″-H and 5″-H), 7.40 (d, H, *J* = 4.3 Hz, 4′-H), 7.70 (d, 2 H, *J* = 9.1 Hz, 2″-H and 6″-H), 7.82 (d, 1 H, *J* = 4.3 Hz, 3′-H), 8.59 (s, 1 H, C=C*H*). ^{13}C NMR (CDCl$_3$) δ 12.4, 12.5, 40.1, 43.0, 43.8, 107.9, 112.0, 120.7, 122.8, 128.3, 134.8, 148.5, 149.2, 151.8, 160.0, 161.4, 164.6, 178.7. Anal. Calcd for $C_{21}H_{23}N_3O_2S_2$: C, 60.99; H, 5.61; N, 10.2; S, 15.5. Found: C, 60.97; H, 5.68; N, 10.1; S, 15.2.

1,3-Diethyl-dihydro-5-((5-(5-phenylthiophen-2-yl)thiophen-2-yl)-methylene)-2-thioxopyrimidine-4,6(1*H*,5*H*)-dione 6a. Pink solid (60%). Mp 240–241 °C. IR(nujol) ν 1685, 1655, 1548, 1528, 1495, 1414, 1328, 1301, 1266, 1171, 1152, 1105, 1073, 998, 972, 897, 806, 782, 759, 722, 759, 722, 691, 640, 608 cm^{-1}. ^{1}H NMR (DMSO-d$_6$) δ 1.22 (m, 6 H, 2 × NCH$_2$C*H*$_3$), 4.42 (m, 4 H, 2 × NC*H*$_2$CH$_3$), 7.43 (m, 3 H, 3″-H, 4″-H and 5″-H), 7.66 (d, 1 H, *J* = 3.9 Hz, 4′-H), 7.77 (m, 3 H, 3′-H, 2″-H and 6″-H), 7.81 (d, 1 H, *J* = 3.9 Hz, 3-H), 8.28 (d, 1 H, *J* = 3.9 Hz, 4-H), 8.64 (s, 1 H,=C*H*). MS (microTOF) *m*/*z* (%): 453 (M$^+$ + 1, 20), 452 (M$^+$, 5), 338 (68), 316 (17), 288 (44), 239 (14), 197 (90), 175 (9). HRMS: *m*/*z* (microTOF) for $C_{23}H_{21}N_2O_2S_3$; calcd 453.07204; found: 453.07597. Anal. Calcd for $C_{23}H_{20}N_2O_2S_3$: C, 61.03; H, 4.45; N, 6.19; S, 21.2. Found: C, 60.78; H, 4.23; N, 6.15; S, 21.3.

5-((5-(5-(4-(Methoxy)phenyl)thiophen-2-yl)thiophen-2-yl)-methylene)-1,3-diethyl-dihydro-2-thioxopyrimidine-4,6(1*H*,5*H*)-dione 6b. Dark blue solid (85%). Mp 263–265 °C. IR(nujol) ν 1685, 1650, 1556, 1531, 1505, 1486, 1170, 1153, 1105, 1026, 974, 897, 824, 815, 803, 794, 783, 751, 722, 668, 648, 632, 582, 550 cm^{-1}. ^{1}H NMR (DMSO-d$_6$) δ 1.22 (m, 6 H, 2 × NCH$_2$C*H*$_3$), 3.80 (s, 3 H, OCH$_3$), 4.45 (m, 4 H, 2 × NC*H*$_2$CH$_3$), 7.00 (d, 2 H, *J* = 8.7 Hz, 3″-H and 5″-H), 7.55 (d, 1 H, *J* = 3.9 Hz, 4′-H), 7.72 (m, 3 H, 2″-H and 6″-H and 3′-H), 7.78 (d, 1 H, *J* = 3.9 Hz, 3-H), 8.27 (d, 1 H, *J* = 3.9 Hz, 4-H), 8.64 (s, 1 H,=C*H*). MS (EI) *m*/*z* (%): 482 (M$^+$, 100), 449 (38), 422 (10), 394 (29), 366 (5), 353 (8), 326 (45), 298 (9), 281 (45), 272 (29), 253 (38), 229 (12), 200 (18), 171 (8), 148 (4). HRMS: *m*/*z* (EI) for $C_{24}H_{22}N_2O_3S_3$; calcd 482.0800; found: 482.0793.

5-((5-(5-(4-(Dimethylamino)phenyl)thiophen-2-yl)thiophen-2-yl)-methylene)-1,3-diethyl-dihydro-2-thioxopyrimidine-4,6(1*H*,5*H*)-dione 6d. Green solid (89%). Mp 246–248 °C. IR(nujol) ν 1682, 1656, 1539, 1513, 1330, 1301, 1258, 1213, 1193, 1151, 1103, 1075, 1059, 1000, 985, 959, 912, 897, 817, 796, 782, 755, 741, 720, 682, 663, 652, 628, 608, 584, 551 cm^{-1}. ^{1}H NMR (DMSO-d$_6$) δ 1.22 (m, 6 H, 2 × NCH$_2$C*H*$_3$), 2.96 (s, 6 H, N(C*H*$_3$)$_2$), 4.45 (m, 4 H, 2 × NC*H*$_2$CH$_3$), 6.75 (d, 2 H, *J* = 8.7 Hz, 3″-H and 5″-H), 7.44 (d, 1 H, *J* = 3.9 Hz, 4′-H), 7.58 (d, 2 H, *J* = 8.7 Hz, 2″-H and 6″-H), 7.68 (d, 1 H, *J* = 4.2 Hz, 3-H), 7.77 (d, 1 H, *J* = 3.9 Hz, 3′-H), 8.25 (d, 1 H, *J* = 4.2 Hz, 4-H), 8.62 (s, 1 H,=CH). MS (microTOF) *m*/*z* (%): 496 (M$^+$ + 1, 45), 495 (M$^+$, 20), 413 (17), 379 (13), 299 (91), 226 (5). HRMS: *m*/*z* (microTOF) for $C_{25}H_{25}N_3O_2S_3$; calcd 495.1109; found: 495.1103. Anal. Calcd for $C_{25}H_{25}N_3O_2S_3$: C, 60.58; H, 5.08; N, 8.48; S, 19.4. Found: C, 60.63; H, 5.08; N, 8.58; S, 18.9.

5-((5-(5-(4-(Diethylamino)phenyl)thiophen-2-yl)thiophen-2-yl)-methylene)-1,3-diethyl-dihydro-2-thioxopyrimidine-4,6(1*H*,5*H*)-dione 6e. Green solid (96%). Mp 199–201 °C. IR(nujol) ν 1684, 1655, 1605, 1551, 1539, 1516, 1481, 1411, 1395, 1356, 1307, 1269, 1250, 1231, 1217, 1159, 1108, 1096, 1077, 1054, 1019, 1001, 956, 925, 900, 881, 861, 813, 793, 779, 740, 735, 720, 680, 651, 625, 610, 584, 547 cm^{-1}. ^{1}H NMR (DMSO-d$_6$) δ 1.10 (t, 6 H, *J* = 6.9 Hz, Ph-N(CH$_2$C*H*$_3$)$_2$, 1.22 (m, 6 H, 2 × NCH$_2$C*H*$_3$), 3.38 (q, 4 H, *J* = 6.9 Hz, PhN(C*H*$_2$CH$_3$)$_2$), 4.44 (m, 4 H, 2 × NC*H*$_2$CH$_3$), 6.71 (d, 2 H, *J* = 8.7 Hz, 3″-H and 5″-H), 7.40 (d, 2 H, *J* = 3.9 Hz, 4′-H), 7.54 (d, 2 H, *J* = 8.7 Hz, 2″-H and 6″-H), 7.68 (d, 1 H, *J* = 3.9 Hz, 3′-H), 7.76 (d, 1 H, *J* = 3.9 Hz, 3-H), 8.25 (d, 1 H, *J* = 3.9 Hz, 4-H), 8.61 (s, 1 H,=C*H*). MS (microTOF) *m*/*z* (%): 524 (M$^+$ + 1, 91), 523 (M$^+$, 31), 497 (24), 453 (30), 393 (32), 349 (27), 318 (16). HRMS: *m*/*z* (microTOF) for $C_{27}H_{30}N_3O_2S_3$; calcd 524.1455; found: 524.1495. Anal. Calcd for $C_{27}H_{29}N_3O_2S_3$: C, 61.92; H, 5.58; N, 8.02; S, 18.4. Found: C, 61.62; H, 5.31; N, 7.99; S, 18.3.

3.2 Photophysical study

All photophysical studies have been performed with freshly-prepared air-equilibrated solutions at room temperature (298 K). UV/Vis absorption spectra of 10^{-5} M chloroform solutions were recorded on a Jasco V-570 spectrophotometer. Steady-state and time-resolved fluorescence measurements were performed on dilute solutions (*ca.* 10^{-6} M, optical density < 0.1) contained in standard 1 cm quartz cuvettes using an Edinburgh Instruments (FLS920) spectrometer in photon-counting mode. Emission spectra were obtained, for each compound, under excitation at the wavelength of the absorption maximum. Fluorescence quantum yields were measured according to literature procedures. Cresyl violet or quinine bisulfate in 1 N H_2SO_4 were used as standards depending on the emission spectral range.[29]

The lifetime values were obtained from the reconvolution fit analysis (Edinburgh F900 analysis software) of decay profiles obtained using the FLS920 instrument under excitation with a nitrogen-filled nanosecond flashlamp. The quality of the fits was evidenced by the reduced χ^2 value ($\chi^2 < 1.1$).

3.3 Two-photon absorption

TPA cross sections (σ_2) were determined from the two-photon excited fluorescence (TPEF) cross sections ($\sigma_2\Phi$) and the fluorescence emission quantum yield (Φ). TPEF cross sections of 10^{-4} M chloroform solutions were measured relative to fluorescein in 0.01 M aqueous NaOH for 715–980 nm,[26] using the well-established method described by Xu and Webb[26a] and the appropriate solvent-related refractive index corrections.[30] Reference values between 700 and 715 nm for fluorescein were taken from the literature.[31] The quadratic dependence of the fluorescence intensity on the excitation power was checked for each sample and all wavelengths, indicating that the measurements were carried out in intensity regimes where saturation or photodegradation did not occur.

Measurements were conducted using excitation sources delivering fs pulses. This is preferred in order to avoid excited state absorption during the pulse duration, a phenomenon which has been shown to lead to overestimated TPA cross-section values. To span the 700–980 nm range, a Nd:YLF-pumped Ti:sapphire oscillator was used generating 150 fs pulses at a 76 MHz rate. To span the 1000–1400 nm range, an OPO (PP-BBO) was added to the setup to collect and modulate the output signal of the Ti:sapphire oscillator. The excitation was focused into the cuvette through a microscope objective (10×, NA 0.25). The fluorescence was detected in epifluorescence mode *via* a dichroic mirror (Chroma 675dcxru) and a barrier filter (Chroma e650sp-2p) by a compact CCD spectrometer module BWTek BTC112E. Total fluorescence intensities were obtained by integrating the corrected emission.

4. Conclusion

The investigation of the photophysical and two-photon absorption properties in a set of series of push–pull derivatives having one or two thienyl moieties in the π-conjugated system allowed us to derive helpful structure–property relationships. These compounds show intense absorption in the near UV-visible region, as well as two-photon absorption in the NIR region in connection with a low-lying intramolecular charge transfer excited state. Interestingly, in such compounds, increasing the length of the conjugated system as well as the strength of the D end-groups allows to *increase* and bathochromically shift both one-photon and two-photon absorption as well as fluorescence. As a result, orange to red bright emitting fluorophores were obtained as illustrated for example in the cases of compounds **3e** (λ_{em} = 612 nm and Φ = 74%) and **4e** (λ_{em} = 727 nm and Φ = 34%) that combine strong NEt_2 donor end-groups and the phenyl–bi(thienyl) conjugated path. In contrast, increasing the strength of the A end-groups also leads to a significant bathochromic shift of both one- and two-photon absorption and emission as well as an increase of (one and two-photon) absorption (ICT) bands but at the cost of a reduction of the fluorescence quantum yield. As a result when combining strong D and A end-groups, fluorophores emitting in the NIR region were obtained as illustrated in the case of fluorophore **6d** (λ_{em} = 781 nm and Φ = 6%).

All push–pull derivatives show a broad TPA band located in the NIR region whose position matches well the intense one-photon absorption band located in the visible region, indicating that the ICT transition is both one and two-photon allowed. This TPA band is significantly red-shifted and its magnitude is found to increase considerably with both the strength of the end-groups and the length of the conjugated path. Such enhancement has also been observed in different quadrupolar and octupolar derivatives with different π-connectors (*i.e.* vinylene–arylene).[11*e*,17*d*–*g*] As a result, push–pull derivatives **4e**, **5d** and **6d** which bear strong D (*i.e.* NR_2) and A (DCV or DETB) end-groups were found to combine large TPA cross-sections in the 1000–1200 nm spectral range (up to 1600 GM), as well as red or NIR emission. These spectral characteristics are of major interest for biological microscopic imaging due to the reduced scattering they provide in tissues. The push–pull arylbithiophenes combining strong D and A end-groups thus hold promise for the design of biphotonic fluorescent probes for bioimaging. We are currently investigating this route.

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