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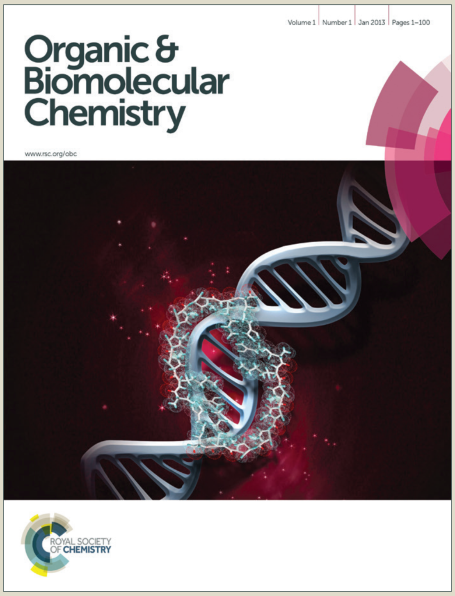

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ARTICLE TYPE

Quadrupolar, emission-tunable π-expanded 1,4-dihydropyrrolo[3,2-*b*]pyrroles – synthesis and optical properties

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The synthesis and optical characterization of six novel heteroaromatic-based chromophores is described. The new dyes present mostly an A-D-A general framework, where A is an electron-deficient aromatic ring and D an electron-rich pyrrole[3,2-*b*]pyrrole moiety, linked *via* triple bonds. It was demonstrated that the increase in molecular length of the chromophore effectively extends π-conjugation. The effect of structural variations on photophysical properties was studied in detail for these compounds and the relationship between the structure and photophysical properties were thoroughly elucidated by comparison with simpler tetraaryl-analogues. The strong charge-transfer character of these functional dyes can be illustrated by large Stokes shifts (4100-7100 cm^{-1}) for A-D-A architectures. The replacement of phenyl rings at positions 2 and 5 with the arylethynylaryl substituents bathochromically shifts both absorption and emission ca. 50-150 nm. The clear dependence of fluorescence maxima on electron-accepting property of the peripheral arylethynyl substituent emphasizes strong π-conjugation in these molecules. The donor-acceptor interactions were also found to influence the two-photon absorbing properties.

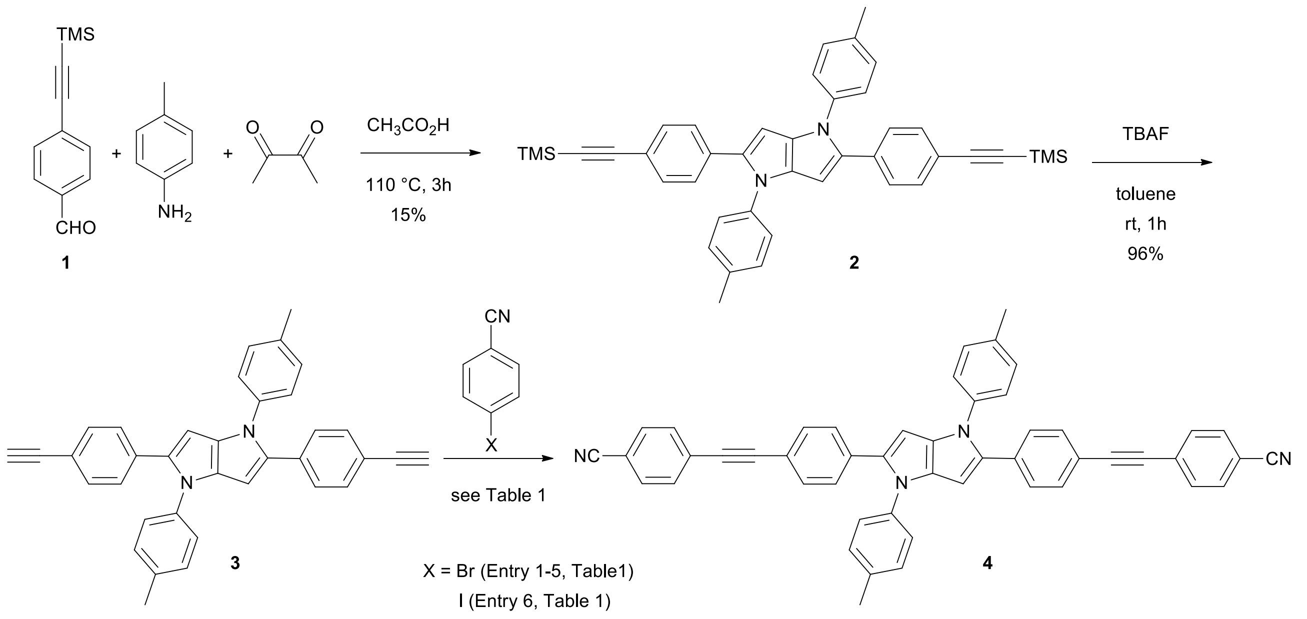
Introduction

Heteropentalenes[1] are a class of 10π-electron aromatic compounds and 1,4-dihydropyrrole[3,2-*b*]pyrrole is the least studied member of this family.[2] In contrast to well explored thieno[3,2-*b*]thiophene, 1,4-dihydropyrrole[3,2-*b*]pyrrole core is the strongest electron-donor among 10π-electron systems. Lack of an efficient synthetic method for the preparation of this skeleton has recently been overcome by the discovery of one-pot domino reaction between aldehydes, primary amines and butane-2,3-dione.[3] The superb optical properties, including intrinsically high fluorescence quantum yields, combined with the straightforward character of the synthesis, make 1,4-dihydropyrrole[3,2-*b*]pyrrole the core unit of choice for application in various areas of photonics, in particular in fluorescence microscopy. Although classical fluorescent microscopy still plays a critical role, two-photon excited fluorescence (TPEF) microscopy can provide better contrast, brighter images and greater detail compared to classical fluorescent microscopy.[4,5] This originates from the fact that the two-photon absorption (2PA) scales quadratically with the intensity of the incident laser radiation, which leads to much higher spatial resolution than could be achieved by one-photon absorption.

Very recently, it has become clear that, for the two-photon technology to realize its full potential, the development of more two-photon-active chromophores that also possess other useful optical or chemical properties, such as high fluorescence quantum yields, easy processability, good photostability, and durability will play a vital role.[4-8] The breakthrough study by Brédas, Marder, Perry and co-workers[9] has revealed that molecules possessing quadrupolar structure (D-A-D, A-D-A), usually possess higher 2PA cross-sections (σ_2) than their dipolar analogs. This is due to the fact that the presence of a donor as the core induces charge transfer from the center to the periphery of the molecule, and hence enables the molecule to display large σ_2 values. Needless to say, the stronger the donor/acceptor ability of corresponding moieties, the higher the values of σ_2.[4,5] We envisioned that the conjugated π-system could be enlarged by two arylethynyl units at each side of 1,4-dihydropyrrole[3,2-*b*]pyrrole core. The intrinsically high electron-donating ability of the pyrrolo[3,2-*b*]pyrrole core, prompted us to perform the study oriented towards the synthesis of A-D-A molecules based on this skeleton.

Results and discussion

Our design implied decoration of the pyrrolo[3,2-*b*]pyrrole skeleton with electron-withdrawing groups linked through C-C triple bonds. Moreover, synthesis of a derivative with terminal methoxy groups was planned as to allow for the direct comparison between influence of electron-rich and electron-poor substituents on optical properties of the chromophore. We reasoned that due to the small dihedral angles between the phenyl ring and the core in analogous 2-phenylindoles (~30 °)[10] the conjugation between the three parts of the desired dyes will be strong. 1,4-Di(4-methylphenyl)-2,5-di(alkynylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (**3**) was prepared in two steps (Scheme 1). Since Sonogashira coupling is the preferred method leading to derivatives of diarylacetylenes,[11] to obtain the designed compounds we decided to synthesize an electron-rich heterocycle bearing two terminal acetylenes and subsequently react it with a range of aryl bromides and aryl iodides. The three component condensation of 4-trimethylsilylethynylbenzaldehyde (**1**),[12] 4-methylaniline and butane-2,3-dione in boiling glacial acetic acid rendered the derivative 1,4-dihydropyrrole[3,2-*b*]pyrrole (**2)** with a 15% yield. Deprotection of TMS group by reaction with tetrabutylammonium fluoride (TBAF) gave product **3**, which served as a platform for planned modifications. The Sonogashira coupling between alkyne **3** and 4-bromobenzonitrile or 4-iodobenzonitrile has been chosen as a model system for the optimization studies (Scheme 1, Table 1). In spite of using diverse and modern protocols[13] we could not reach a higher yield of product **4** than 24%.



Scheme 1.

Table 1. Optimisation of Sonogashira reaction leading to compound **4**

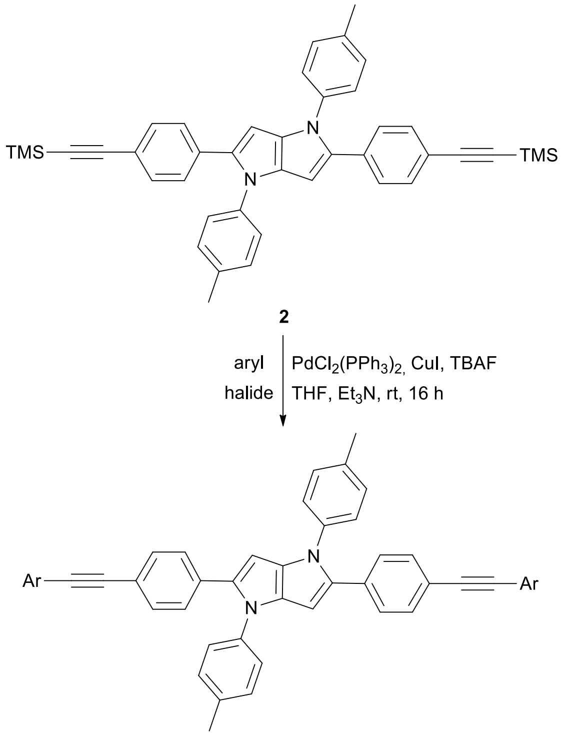
Entry	Reaction conditions	% yield
1	Pd(PPh$_3$)$_4$, CuI, Et$_3$N THF, 70 ºC, 16 h	24
2	Pd(PhCN)$_2$Cl$_2$, P(*t*-Bu)$_3$, CuI HN(*i*-Pr)$_2$, dioxane, rt, 16 h	0
3	Pd$_2$dba$_3$, AsPh$_3$, Et$_3$N toluene, rt/80 ºC, 24 h	20
4	Cs$_2$CO$_3$, CuI, DABCO dioxane, 135 ºC, 16 h	0
5	Pd$_2$(dba)$_3$, P(*t*-Bu)$_3$, Et$_3$N THF, rt, 16 h	9
6	Cs$_2$CO$_3$, CuI, phenanthroline, dioxane, 135 ºC, 16h	10

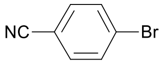
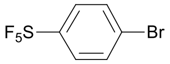
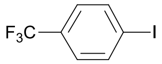
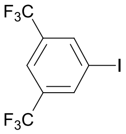
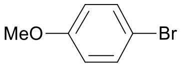
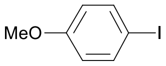
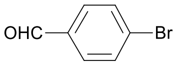
Looking for new strategies we directed our research efforts towards sila-Sonogashira coupling.[14] The combination of both deprotection and coupling in one step, under conditions described by Henze *et al*[15] allowed us to obtain compound **4** in higher yield with concomitant shortening of the whole procedure (Table 2). Encouraged by this finding, we subsequently investigated whether this synthetic route is applicable for other iodoarenes and bromoarenes.

Subjecting the mixture of 2,6-di(4-(trimethylsilylethynyl)phenyl)-1,5-di(4-methylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (**2**) and aryl halides bearing various substituents to sila-Sonogashira coupling conditions gave products **4-8** in yields ranging from 15 to 56%. It is noteworthy that although reaction proceeds for both electron-poor and electron-rich halides, 4-bromobenzonitrile was the most efficient substrate in this reaction. Still, compound **8** could only be obtained when the process was performed with 4-iodoanisole rather than 4-bromoanisole (Entries 5 and 6, Table 2). Interestingly, reaction of 4-bromobenzaldehyde with compound **2** led to a broad range of side products which impeded the purification process and caused loss in overall yield of derivative **9**. This unsatisfactory result prompted us to change the reaction conditions and employ standard Sonogashira coupling (see Table 1, Entry 1), this increased the yield of the product **9** to 56%.

Spectral characteristics of products **4-9** were then examined and compared to those of parent compounds **10** and **11**[3] (Fig. 1-3, Table 3). The absorption spectra of these dyes have broad bands located between 350 and 500 nm (Fig. 2 and 3). The molar absorption coefficients are in the range 50000-70000 regardless the electronic character of the substituents i.e. they are significantly higher than for previously reported tetraaryl-pyrrolo[3,2-*b*]pyrroles.[3] The characteristic feature was the bathochromic shift of absorption when going from compounds **10-11** to dyes **4-9** (~50 nm). This strong bathochromic shift proves the existence of electronic communication in the ground state throughout the whole molecule. In typical low viscosity solvent such as CH$_2$Cl$_2$, the rotation of the benzene ring along the linker is not disturbed, and coplanarity between the substituted phenyl ring and pyrrolo[3,2-*b*]pyrrole the core is attained.

Table 2. Results of one-pot sila-Sonogashira coupling reaction



Entry	Aryl halide	Product #	Yield (%)
1		**4**	56
2		**5**	21
3		**6**	15
4		**7**	33
5		**8**	0
6		**8**	30
7		**9**	Traces[a]

[a] Due to unsatisfactory result we decide to change reaction conditions and employ standard Sonogashira reaction conditions (see Table 1, Entry 1) what increased the yield of the product **9** to 56%.

All of the π-expanded pyrrolo[3,2-*b*]pyrroles are fluorescent. The color of fluorescence of **4**-**9** ranges from blue, turquoise to orange. Fluorescence maxima, except for compound **8**, are shifted above 500 nm. The fluorescence maxima (λ_{max}^{fl}) of the series **4**-**9** are gradually red-shifted upon increasing strength of electron-withdrawing group. The strong relationship between electron-withdrawing strength of the substituent and the λ_{max}^{fl} supports the existence of strong electronic communication in these molecules in the excited state. Fluorescence quantum yields of products **4**-**8** were found to be moderate to good (Φ_{fl} = 16-53%) and the highest fluorescence quantum yield (53%) was measured for **8**. Product **9** had the lowest quantum yield (2%), which is quite typical for aldehydes. The fluorescence quantum yield and Stokes shift of **6** are similar to those of compound **7**, while dialdehyde **9** possesses very high Stokes shift (7000 cm^{-1}). The stronger electron-withdrawing substituents in **9** (CHO) and **4** (CN) compared to that in **6** and **7** (CF_3) confer further bathochromic shift of absorption and emission as expected for push-pull chromophores.[16] Large Stokes shift corresponds to significant change of geometry between ground and excited states. Dyes **4**-**9** compare very favorably with quadrupolar dyes of similar complexity.[17]

Two-photon absorption has been measured using TPFM and results are shown in Table 3 and on Figs. 4 and 5. The measurements were conducted in the 700-1020 nm range (corresponding to the relevant biological spectral window). In the spectral range of interest, two-photon absorption cross-sections are not strongly dependent on the magnitude of charge-transfer. Indeed, strongly electron-donating 4-methoxyphenyl substituent gave relatively intense 2PA signal. Interestingly two-photon absorption of these quadrupolar molecules is typically within 100-500 GM at 720 nm.

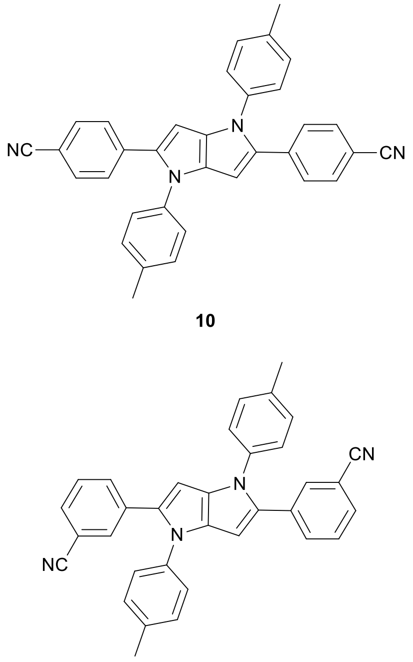


Fig 1. Structures of pyrrolo[3,2-*b*]pyrroles **10** and **11**.

Indeed the highest value in the NIR region of the spectrum can be attributed to compound **7** possessing two 3,5-bis(trifluoromethyl) substituents while strongly electron-withdrawing **4** and **9** have lower σ_2 (Table 3, Fig. 4). On the other hand, it is clear that, tetraaryl-derivative **10** possessing two 4-cyanophenyl substituents at positions 2 and 5 also behaves like A-D-A system (σ_2~300

GM). Eventually, the critical figure of merit i.e. two-photon brightness is the highest for dye **7** (~200 GM). As illustrated in Figs. 4-5 for compounds **4-11**, the lowest (strongly one-photon allowed) excited state is moderately intensive, and the higher (only weakly one-photon allowed) state, responsible for the large 2PA response is located below 700 nm. This feature, is reminiscent of the behavior of symmetrical quadrupolar derivatives.[18]

Table 3. Photophysical data for **4-11** in CH_2Cl_2

Product #	λ_{abs}/nm	λ_{em}/nm	$\Phi_{fl}{}^{a}$(%)	$\sigma_{2(720\ nm)}$ (GM)	*Stokes shift* (cm^{-1})
4	428	549	22	250	5200
5	418	523	16	5	4800
6	414	511	42	800	4600
7	421	522	37	500	4600
8	401	479	53	100	4100
9	434	629	2	300	7100
10[b]	406	461	88	260	2900
11[b]	368	462	17	25	5500

[a] Determined with quinine sulphate in H_2SO_4 (0.5 M) as a standard.
[b] Ref. 3.

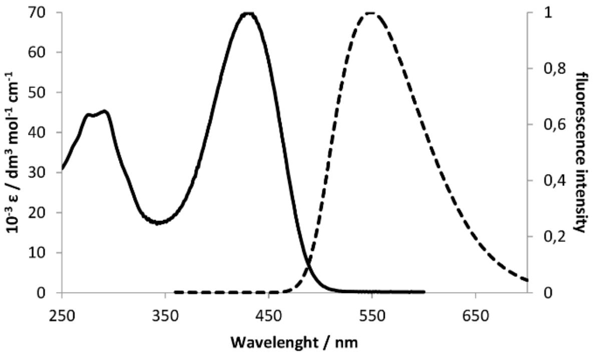


Fig.2. Absorption (—) and normalized fluorescence (- - -) spectra of **4** in CH_2Cl_2.

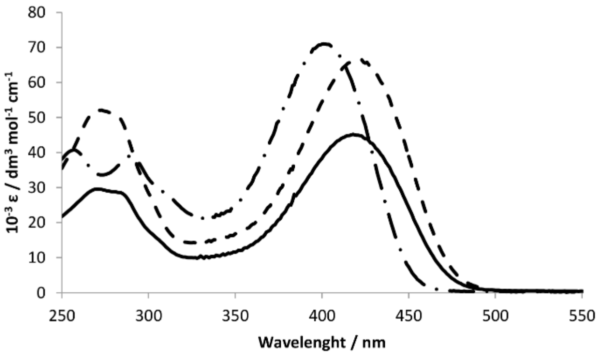


Fig.3 Absorption spectra of **5** (—), **7** (- - -), and **8** (- · -) in CH_2Cl_2.

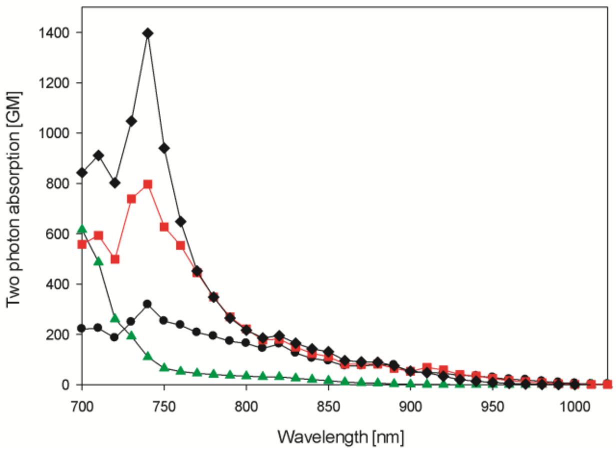


Fig.4. Two-photon absorption **4** (black dots), **6** (black diamonds), **7** (red squares), **10** (green triangles) in CH_2Cl_2.

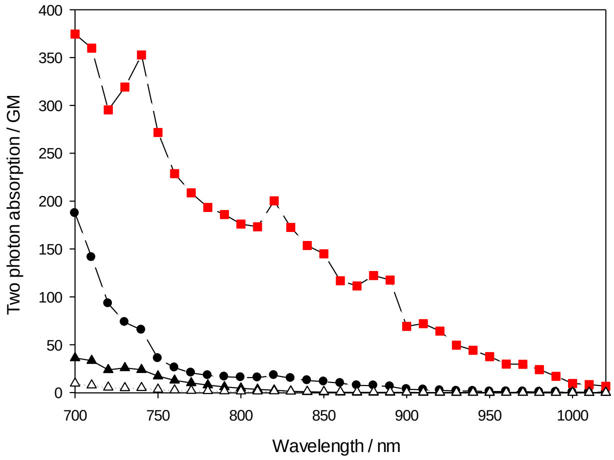


Fig.5 Two-photon absorption spectra of **8** (black dots), **9** (red squares), **5** (open triangles) and **11** (black triangles) in CH_2Cl_2.

Conclusions

In summary, the first examples of π-expanded pyrrolo[3,2-*b*]pyrroles have been described.[X] It has been proven, that only 'sila version' of Sonogashira coupling is an efficient method to assemble these molecules. The proposed synthetic method is operationally, simple and leads to new functional dyes possessing strong absorption in violet-blue region combined with reasonably intensive blue-green fluorescence. In sharp contrast to previously described tetraarylpyrrolo[3,2-*b*]pyrroles both absorption and emission are bathochromically shifted. Thus, it can be reasonably concluded that the connection with arylethynylaryl groups at the 2,5-positions of pyrrolo[3,2-*b*]pyrroles extends π-conjugation effectively relative to that of 1,2,4,5-tetraarylpyrrolo[3,2-*b*]pyrroles. The most promising dye bearing two 3,5-$(CF_3)_2C_6H_3$ groups possesses appreciable two-photon brightness (~200GM) while its calculated Stokes shift remains relatively large (4600 cm^{-1}). A new and efficient turquoise emitter, namely the compound possessing two 4-methoxyphenylethynylphenyl substituents was identified. Our studies illustrate the effect of different substituents on the electronic properties of the pyrrole[3,2-*b*]pyrroles and reveal that both absorption and emission can be tuned by straightforward structural manipulations. These results are not only of theoretical significance in that they provide insight into factors influencing the electronic structure of extended π-systems, but they may also open doors to practical applications in such diverse areas as molecular electronics and fluorescent imaging.

Experimental section

All chemicals were used as received unless otherwise noted. Reagent grade solvents (CH_2Cl_2, hexanes) were distilled prior to use. All reported NMR spectra were recorded on 400, 500 MHz and 600 MHz spectrometer. UV-vis absorption and fluorescent spectra were recorded in acetonitrile. Chromatography was performed on silica (200-400 mesh) and dry column vacuum chromatography (DCVC) was performed on preparative thin layer chromatography silica (Merck 107747). Mass spectra were obtained via EI-MS and FD-MS.

Synthesis

1,4-Di-(4-methylphenyl)-2,5-bis(4((trimethylsilyl)ethynyl) phenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (2)

4-Methylaniline (1.27 g, 12 mmol) and 4-(trimethylsilylethynyl)benzaldehyde (2.4 g, 12 mmol) were stirred in glacial acetic acid (10 mL) at 100 °C for 30 min. Then butane-2,3-dione (519 µl, 6 mmol) was added and the resulting mixture was stirred at 100 °C for 3 h. After cooling the precipitate was filtered off and washed with glacial acetic acid. Recrystallization from EtOAc afforded pure product as a yellow solid in 15% yield (567 mg). $R_f = 0.78$ (SiO_2, hexane/CH_2Cl_2, 1:1). Mp 314.0-314.3 °C (decomposition). ^1H NMR (500 MHz, $CDCl_3$) δ 7.26 (AA'XX', 4H), 7.14 (m, 12H), 6.36 (s, 2H), 2.37 (s, 6H), 0.23 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.0, 135.9, 132.0 (2), 130.0, 129.4, 129.1, 128.2, 127.8 (2), 125.3, 106.6, 94.9, 21.2, 0.14. HRMS (EI+) calcd for $C_{42}H_{42}N_2Si_2$: 630.2905 [M$^+$], found: 630.2905. λ_{abs} (CH_2Cl_2, $\varepsilon \times 10^{-3}$) 393 (60) nm.

2,5-Bis(4-ethynylphenyl)-1,4-di-(4-methylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (3)

TMS protected derivative **2** (567 mg, 0.90 mmol) was dissolved in THF (5 ml) and TBAF (588 mg, 2.25 mmol) was added. Reaction mixture was stirred for 3 h in rt. Solvent was evaporated and crude product was recrystallized from EtOAc. Pure product was obtained as a yellow solid in 96% yield (420 mg). $R_f = 0.67$ (SiO_2, hexane/CH_2Cl_2, 1:1). Mp 290.0-290.3 °C (decomposition). ^1H NMR (500 MHz, $CDCl_3$) δ 7.33 (AA'XX', 4H), 7.16 (m, 8H), 7.14 (AA'XX', 4H), 6.39 (s, 2H), 3.07 (s, 2H), 2.38 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.4, 135.9, 135.6, 134.2, 132.6, 132.1, 130.0, 127.8, 125.3, 119.5, 95.0, 84.0, 77.6, 21.2. HRMS (EI+) calcd for $C_{36}H_{26}N_2$: 486.2096 [M$^+$], found: 486.2100. Anal. calcd for $C_{36}H_{26}N_2$: C, 88.86; H, 5.39; N, 5.76; found: C, 88.70; H, 5.31; N, 5.77. λ_{abs} (CH_2Cl_2, $\varepsilon \times 10^{-3}$) 387 (53) nm.

Representative procedure for sila-Sonogashira coupling:

2,5-Di(4-(4-cyanophenylethynyl)phenyl)-1,4-di(4-methylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (4)

The mixture of 1,4-di-(4-methylphenyl)-2,5-bis(4-((trimethylsilyl)ethynyl)phenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (**2**, 1.20 mg, 3.17×10^{-5} mol), $PdCl_2(PPh_3)_2$ (2.2 mg, 3.17×10^{-6} mol), CuI (0.6 mg, 3.17×10^{-6} mol) and 4-bromobenzonitrile (12 mg, 6.317×10^{-5} mol) in dry THF (0.5 ml) with Et_3N (0.5 ml, 3.6 mmol) were deoxygenated by freeze-pump-thaw cycles and purged with argon gas in an oven dried Schlenk flask. TBAF (21 mg, 7.92×10^{-6} mol) was added, and the reaction mixture was stirred for 16 h at rt under an argon atmosphere. The crude mixture was filtered through celit and the solvent was distilled off. Purification using DCVC method (SiO_2, hexane/CH_2Cl_2, 4:1) afforded pure product as a yellow solid in 56% yield (12 mg). $R_f = 0.27$ (SiO_2, hexane/CH_2Cl_2, 1:1). Mp 313.2-313.5 °C (decomposition). ^1H NMR (500 MHz, $CDCl_3$) δ 7.61 (AA'XX', 4H), 7.57 (AA'XX', 4H), 7.38 (AA'XX', 4H), 7.21 (AA'XX', 4H), 7.19 (m, 8H), 6.42 (s, 2H), 2.39 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.5, 136.1, 135.7, 134.5, 132.9, 132.2, 132.1, 131.8, 130.1, 128.5, 127.9, 125.4, 119.6, 118.6, 111.5, 95.2, 88.5, 21.2. HRMS (EI+) calcd for $C_{50}H_{32}N_4$: 688.2627 [M$^+$], found: 688.2596. λ_{abs} (CH_2Cl_2, $\varepsilon \times 10^{-3}$) 428 (70) nm.

2,5-Di(4-(4-pentafluorothiophenyl)ethynylphenyl)-1,4-di(4-methylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (5)

Purification using DCVC method (SiO_2, hexane/CH_2Cl_2, 4:1) afforded pure product as a yellow solid in 21% yield (6 mg). $R_f = 0.72$ (SiO_2, hexane/CH_2Cl_2, 1:1). Mp 207.0-207.3 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 7.65 (AA'XX', 4H), 7.48 (AA'XX', 4H), 7.33 (AA'XX', 4H), 7.14 (AA'XX', 4H), 7.13 – 7.08 (m, 8H), 6.36 (s, 2H), 2.32 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.3, 135.9, 135.5, 134.2, 132.6, 131.6, 131.5, 129.9, 127.7, 127.1, 126.0, 125.2, 119.5, 95.0, 92.7, 87.9, 29.7, 21.0. HRMS (EI+) calcd for $C_{48}H_{32}F_{10}N_2S_2$: 890.1847 [M$^+$], found: 890.1843. λ_{abs} (CH_2Cl_2, $\varepsilon \times 10^{-3}$) 418 (45) nm.

2,5-Di(4-(4-trifluoromethyl)ethynylphenyl)-1,4-di(4-methylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (6)

Purification using DCVC method (SiO_2, hexane/CH_2Cl_2, 6:1) afforded pure product as an orange solid in 15% yield (4 mg). $R_f = 0.76$ (SiO_2, hexane/CH_2Cl_2, 1:1). Mp 346.9-347.2 °C (decomposition). ^1H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 8H), 7.39 (AA'XX', 4H), 7.19 (m, 12H), 6.42 (s, 2H), 2.38 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.5, 136.0, 131.9 (2), 131.7, 130.1, 129.8, 127.9 (2), 127.3 (2), 125.5 (2), 125.4, 125.4, 123.1, 112.6, 95.1, 21.2. HRMS (EI+) calcd for $C_{50}H_{32}F_6N_2$: 774.2470 [M$^+$], found: 774.2461. λ_{abs} (CH_2Cl_2, $\varepsilon \times 10^{-3}$) 414 (62) nm.

2,5-Di(4-(3,5-di(trifluoromethyl)ethynylphenyl)-1,4-di(4-methylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (7)

Purification using DCVC method (SiO_2, hexane/CH_2Cl_2, 8:1) afforded pure product as a yellow solid in 33% yield (10 mg). $R_f = 0.78$ (SiO_2, hexane/CH_2Cl_2, 1:1). Mp 316.6-316.9 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 7.92 (s, 4H), 7.80 (s, 2H), 7.40 (AA'XX', 4H), 7.23 (AA'XX', 4H), 7.20 (m, 8H), 6.45 (s, 2H), 2.39 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.4, 136.1, 134.6, 132.9, 132.2, 132.0, 131.8, 131.4, 130.1, 127.9, 125.9, 125.3, 124.2, 122.0, 119.1, 95.2, 93.3, 87.0, 21.2. HRMS (EI+) calcd for $C_{52}H_{30}F_{12}N_2$: 910.2215 [M^+], found: 910.2188. λ_{abs} (CH_2Cl_2, $\varepsilon \times 10^{-3}$) 421 (67) nm.

2,5-Di(4-(methoxy)ethynylphenyl)-1,4-di(4-methylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (8)

Purification using DCVC method (SiO_2, hexane/CH_2Cl_2, 3:1) afforded pure product as a yellow solid in 30% yield (7 mg). R_f = 0.44 (silica, hexane/CH_2Cl_2, 1:1). Mp 315.3-315.6 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 7.44 (AA'XX', 4H), 7.35 (AA'XX', 4H), 7.18 (s, 12H), 6.87 (AA'XX', 4H), 6.40 (s, 2H), 3.82 (s, 6H), 2.38 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.7, 137.5, 135.8, 133.1, 133.0, 131.3 (2), 130.0, 129.2, 128.4, 127.8 (2), 125.8, 125.3, 114.1, 95.0, 55.5, 21.6, 21.2. HRMS (EI+) calcd for $C_{50}H_{38}N_2O_2$: 698.2933 [M^+], found: 698.2955. λ_{abs} (CH_2Cl_2, $\varepsilon \times 10^{-3}$) 401 (71) nm.

2,5-Di(4-(formylethynyl)phenyl)-1,4-di(4-methylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (9)

The mixture of 4-bromobenzaldehyde (50 mg, 2.71×10^{-4} mol), 2,5-bis(4-ethynylphenyl)-1,4-di-(4-methylphenyl)-1,4-dihydropyrrolo[3,2-*b*]pyrrole (**3**, 66 mg, 1.36×10^{-4} mol), $Pd(PPh_3)_4$ (7 mg, 6.06×10^{-6} mol), CuI (2 mg, 1.06×10^{-5} mol) in THF (2 ml) with Et_3N (120 μl, 0.86 mmol) were deoxygenated by freeze-pump-thaw cycles in a Schlenk flask and stirred in 70 °C for 16 h. The crude mixture was filtered through celit and the solvent was distilled off. Purification using DCVC method (SiO_2, hexane/CH_2Cl_2, 1:1) afforded pure product as an orange solid in 56% yield (31 mg). R_f = 0.16 (SiO_2, hexane/CH_2Cl_2, 1:1). Mp 283.3-283.6 °C (decomposition). ^1H NMR (500 MHz, $CDCl_3$) δ 10.01 (s, 2H), 7.85 (AA'XX', 4H), 7.64 (AA'XX', 4H), 7.40 (AA'XX', 4H), 7.19 (s, 12H), 6.43 (s, 2H), 2.39 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.5, 137.4, 136.0, 135.5, 132.1, 131.8 (2), 130.1, 129.7, 127.8 (2), 125.3, 120.0, 110.1, 94.9, 93.9, 89.5, 29.7, 21.2. HRMS (EI+) calcd for $C_{50}H_{34}N_2O_2$: 694.2580 [M^+], found: 694.2585. λ_{abs} ($CHCl_3$, $\varepsilon \times 103$) 434 (30) nm.

Two photon fluorescence intensity measurements

Dichloromethane (CHROMASOLV®, for HPLC, ≥99.9%), methanol (CHROMASOLV®, for HPLC, ≥99.9), 5-Carboxyfluorescein (99% (HPLC)), Rhodamine B and Rhodamine 6G were purchased from Sigma-Aldrich, Denmark. The samples were dissolved in dichloromethane, Rh B and Rh 6G were dissolved in methanol. The fluorescein was dissolved in a CAPS buffer (N-cyclohexyl-3-aminopropanesulfonic acid) pH 11. The concentrations of the samples and references where determined by a dilution series in a spectrophotometer (Perkin Elmer lambda 35) using quartz cuvettes. The single photon fluorescence emission spectra were measured using a spectrofluorometer (ChronosFD from ISS, Champaign, IL USA). The two photon excited emission spectra were collected using a custom built multiphoton excitation spectrofluorometer similar to the previously used.[19] Briefly, the excitation source was a Ti:Sa laser (HPeMaiTai DeepSee, Spectra Physics, Mountain View, CA). The laser power was controlled using motorized halfwave plate together with a polarizer. The excitation light was focused into the sample using a 60x super long working objective (Nikon) The emission was collected through the objective and passed through a Multiphoton-Emitter HC 680/SP (AHF analysentechnik AG, Tuebingen, Germany) to a multimode optical fiber (M200L02S-A, Thorlabs Sweden AB Goteborg, Sweden). The emission was then sent through a monochromator (ARC-SP2155, BFi OPTiLAS, Sweden) and the spectra were imaged by a cooled CCD camera (PIXIS 400B, Princeton Instruments - New Jersey, USA). A motorized XY microscope stage (Nikon) was used as a sample holder. The laser, laser power, camera and XY stage was controlled using ImageJ[20] and custom scripts. The relative laser power was monitored by a power meter (PM100D with a S142C head, Thorlabs Sweden AB Goteborg, Sweden). Calculations were done using custom Matlab code (MathWorks, Natick, USA). The 2-photon absorption spectra were measured relative to standard fluorophores, with well characterized spectra.[21] The measurement of the sample and standard under the same conditions allows for correction of changes of the temporal pulse profile and spatial beam profile of the laser at different wavelengths. To eliminate possible artifacts, due to photo bleaching or linear absorption, we checked that the fluorescence signal increased as the square of the excitation intensity at the different excitation wavelengths for the different samples. The absolute 2-photon absorption cross sections were calculated using a relative fluorescence intensity technique as described previously.[21] Flourescein in a CAPS buffer, (pH11), Rh 6G and Rh B in methanol were used to reference the system. The measurements for the two photon cross-sections were carried out at least five times for the different samples with excitation wavelengths from 700nm to 1020nm.

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Notes and references

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Graphical abstract:

A-D-A chromophores containing pyrrolo[3,2-*b*]pyrrole, as central donor moiety, display blue, turquoise, yellow and orange fluorescence, depending on the strength of electron-withdrawing substituent.