Towards "smart" multiphoton fluorophores: strongly solvatochromic probes for two-photon sensing of micropolarity

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New fluorophores, combining broad, very high two-photon absorption in the near-infrared region with a marked dependence of their emission spectra on solvent polarity, have been designed as model probes for two-photon sensing of the chemical environment.

Molecular two-photon absorption (TPA) has attracted a lot of interest over recent years in correlation with the many applications it offers, both in biological imaging and materials science. These include two-photon laser scanning microscopy imaging (TPLSM), 3D optical data storage, localised photodynamic therapy, microfabrication and optical power limitation.[1] This has driven the designing of compounds displaying enhanced TPA cross-sections along with specific features, depending on their specific application. Whereas a number of one-photon excitable medium-sensitive probes and highly solvatochromic fluorophores have been developed because of their numerous potential applications,[2] medium-sensitive, two-photon excited fluorescence (TPEF) probes are still largely undeveloped, despite TPEF fluorophores sensitive to ions having recently begun to appear.[3–5] Fluorescent molecules having very high two-photon absorptivities and showing strongly medium-sensitive luminescence would provide medium-responsive TPA fluorophores as micropolarity probes for various media of interest.

With this in mind, we have implemented a molecular engineering strategy towards fluorophores showing broad and intense TPEF in the spectral region of interest for applications (red–NIR region), with a marked dependence of their photoluminescence (PL) characteristics on the solvent polarity. A number of solvatochromic, one-photon fluorescent probes of the donor–acceptor (D–A) type have been reported.[2,6] These push–pull molecules, such as Prodan, Nile Red, 7-aminocoumarins and Fluoroprobe, exhibit intramolecular charge transfer phenomena† and possess polar emissive states, whose emission is highly dependent on solvent polarity. However, most dipolar chromophores do not exhibit very high two-photon absorption cross-sections[4,7,8] compared to quadrupolar and octupolar derivatives which do.[5,9,10]

Recently, a joint experimental and theoretical study revealed that combining dipolar branches within a three-branched octupolar structure[11] by means of a common electron-donating triphenylamine core (D), resulted in excitonic coupling between the dipolar branches (D–A). This induced an intense TPA band, with the emission originating from a dipolar emissive excited-state, localised on one branch‡ (Fig. 1). Based on this observation, our strategy was to modify the octupolar triphenylamine derivatives to increase the excitonic coupling between the branches (so as to increase the TPA efficiency[12]) by increasing the transition dipole of the branches, and to favor the formation of a highly polar emissive excited-state (so as to enforce strongly polarity-dependent emission). To do so, we have combined elongated arms, built from arylene–vinylene moieties, with the powerful electron-withdrawing groups SO_2CF_3 (Hammett's $\sigma_p = 0.96$) or $SO_2C_4F_9$ (Hammett's

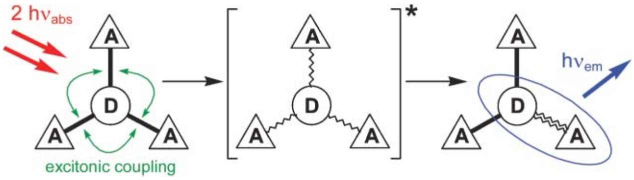
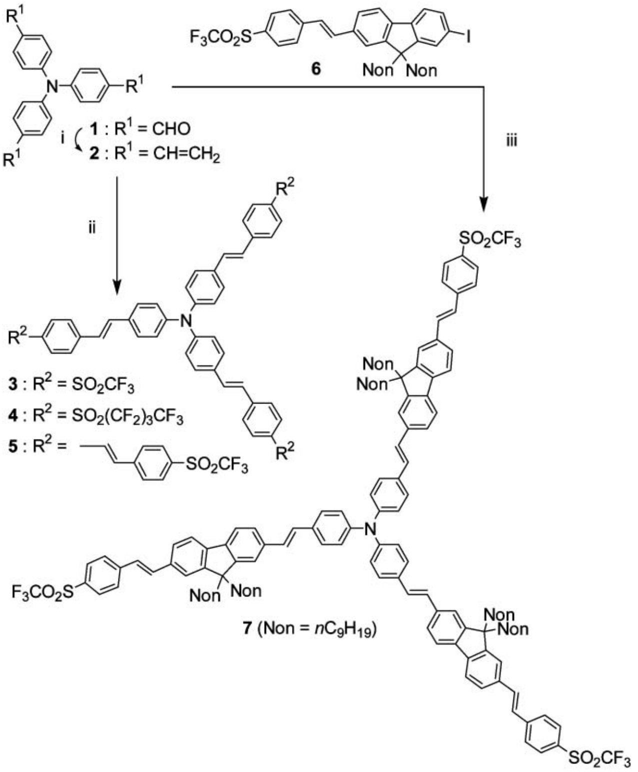


Fig. 1 Octupolar fluorophores for enhanced TPA (excitonic coupling) and localised emission from a highly solvatochromic dipolar branch.



Scheme 1 Synthesis of octupolar fluorophores. i: methyltriphenylphosphonium iodide, NaH, THF, 20 °C, 60 h (yield of **2**, 43%); ii: **2** (1 equiv.), R^2–Ph–X (4 equiv.), $Pd(OAc)_2$, $P(o\text{-tolyl})_3$, DMF/Et_3N, 100 °C (yield of **3**, 76%; **4**, 55%; **5**, 31%); iii: **2** (1 equiv.), **6** (4 equiv.), $Pd(OAc)_2$, $P(o\text{-tolyl})_3$, DMF/Et_3N, 100 °C (yield of **7**, 18%). Non = *n*-nonyl.

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Table 1 Photophysical data for fluorophores **3–5** and **7** in toluene

	λ_{abs}/nm	ε/M^{-1} cm^{-1}	λ_{em}/nm	Stokes shift/10^3 cm^{-1a}	Φ^b	τ/ns^c	Normalized solvatochromic shift/10^3 cm^{-1d}	λ_{TPA}(max)/nm	σ_2 at λ_{TPA}(max)/GM^e
3	430	80800	494	3.0	0.71	1.85	13.1	740	1340
4	435	79000	499	3.0	0.77	1.84	15.5	755	1430
5	440	128000	517	3.4	0.84	1.51	20.8	800	2070
7	435	119000	498	2.9	0.93	1.29	23.2	740	2080

[a] Stokes shift = $(1/\lambda_{abs} - 1/\lambda_{em})$. [b] Fluorescence quantum yield in toluene determined relative to Fluorescein in 0.1 M NaOH. [c] Experimental fluorescence lifetime measured by time-correlated single photon counting. [d] Value of the slope derived from the linear dependence of the Stokes shift on the polarity–polarizability function (Δf) of the solvent ($\Delta f = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1)$) where ε is the dielectric constant and n the refractive index). [e] 1 GM = 10^{-50} cm^4 s photon^{-1}.

$\sigma_p = 1.09$) (Scheme 1), the aim being that they would act as good conduits for long distance intramolecular charge transfer. These trigonal chromophores were synthesised by means of a threefold Heck coupling of trivinyl **2** with its corresponding halo derivative (Scheme 1).

All newly synthesised fluorophores combine a high fluorescence quantum yield (Table 1) and broad TPA bands in the 700–1000 nm spectral region (Fig. 2); a region of application interest because of increased penetration depth in scattering media and the availability of suitable excitation sources.§ We note that the TPA maxima occur at less than half the wavelength of the one-photon maximum wavelength, indicating that there is significant excitonic coupling between the dipolar branches.[11]

As indicated by comparing chromophores **3** and **4**, increasing the peripheral group's acceptor strength results in a red shift and enhancement of the TPA bands. Increasing the size of the compounds leads to a further marked TPA enhancement of the peak, and to a definite broadening of the TPA spectrum in the desired spectral range. As a result, fluorophore **7**, whose TPEF action cross-section ($\sigma_2\Phi$) peaks at 1940 GM (740 nm), maintains a very high action cross-section both at 800 nm (1500 GM) and 900 nm (950 GM). To the best of our knowledge, this is the first example of a dye maintaining such a high TPEF across the whole 700–900 nm spectral range, offering much greater flexibility in terms of excitation sources. In contrast, the TPEF cross-section of coumarin-307, a typical dipolar fluorophore, peaks at 16 GM (800 nm).[8]

Moreover, the PL of the octupolar fluorophores is highly solvent sensitive, demonstrating that the engineering strategy is valid. As illustrated in Fig. 3 for molecule **5**, a marked bathochromic shift of the emission band is observed with increasing solvent polarity. In contrast, the absorption is little affected.¶ This suggests that an intramolecular charge transfer phenomenon takes place after excitation, prior to emission, and that the emission stems from a strongly dipolar emissive state. Indeed the solvatochromic behaviour was found to follow a Lippert–Mataga relationship;[13] a linear dependence of the Stokes shift as a function of the polarity–polarizability function, Δf, being observed (Table 1). Increasing the acceptor strength lead to an increase in the solvatochromism. Interestingly, further amplification of the PL sensitivity was achieved by lengthening the conjugated arms of fluorophores **5** and **7**, leading to normalized solvatochromic shifts larger than 20 000 cm^{-1}. Such PL sensitivity has previously only been reported for smaller D–A fluorophores,[6] and never before for fluorophores with strong two-photon absorption. This marked sensitivity of the PL on the micropolarity, in combination with their high two-photon absorption cross-section in the 700–950 nm spectral range, makes these derivatives promising environment-sensitive TPEF probes; in particular, for applications that would benefit from reduced scattering and background fluorescence (*e.g.* originating from endogeneous chromophores[14]). Two-photon excitation thus offers both an enhanced selectivity for fluorescence signals coming from the probes and an intrinsic 3D resolution not allowed by one-photon excitation.

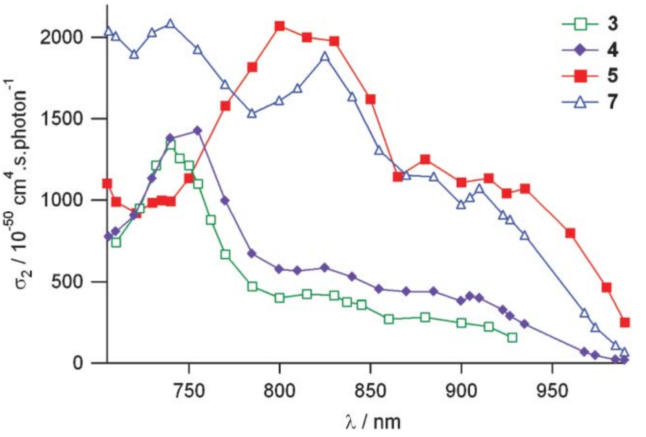

Fig. 2 TPA spectra of fluorophores **3–5** and **7** determined by femtosecond TPEF measurements in toluene.

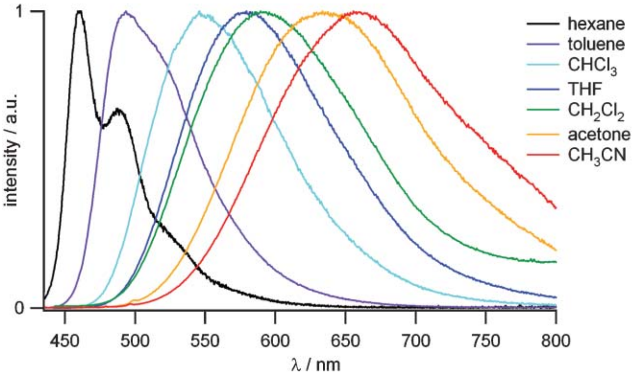

Fig. 3 Solvatochromic behaviour of fluorophore **3** (normalized one photon-induced emission spectra). Quantum yields: 0.40 (hexane), 0.71 (toluene), 0.89 (CHCl$_3$), 0.79 (THF), 0.88 (CH$_2$Cl$_2$), 0.12 (acetone), 0.04 (CH$_3$CN).

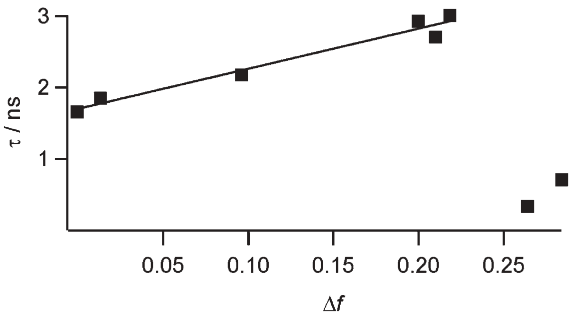

Fig. 4 Variation of the fluorescence lifetime of fluorophore **3** as a function of the solvent polarity–polarizability.

The fluorescence lifetimes also vary as a function of the environment. As illustrated in Fig. 4 for fluorophore **3**, a linear dependence of the fluorescence lifetime on the polarity–polarizability function is observed in a range of apolar and medium polarity solvents. This quasi-linear dependence is lost in more polar solvents as a result of a drastic increase in the rate of non-radiative decay. This is also responsible for the decrease of the fluorescence quantum yield in polar solvents, a feature shared with most dipolar fluorescent probes.

In conclusion, we have implemented a successful strategy that leads to two-photon fluorescent probes which combine intense two-photon absorption over a broad range of wavelengths (700–900 nm) and have PL characteristics (emission spectra and to a lesser extent fluorescence lifetime) that are strongly dependent on their environment. Such fluorophores can be useful for sensing in various chemical environments, including scattering media. These fluorophores may also be derivatised to yield two-photon fluorescent labels for environment sensing in materials such as silica and zeolites.

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Notes and references

† In some cases twisted intramolecular charge transfer.

‡ Such excitation localization phenomena have been demonstrated for several branched structures.[15]

§ The TPA spectra were determined by investigating their TPEF in toluene, according to the experimental protocol established by Xu and Webb,[8] using femtosecond excitation pulses. This experimental protocol avoids the contribution of excited-state absorption, known to lead to artificially enhanced TPA cross-sections.

¶ The TPA spectrum was also found to be similar (within experimental error) in a more polar solvent.

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