Synthesis of chromophores combining second harmonic generation and two photon induced fluorescence properties†

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The design of new chromophores presenting simultaneous SHG and TPEF properties is reported.

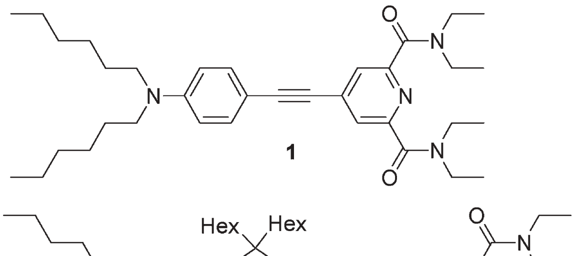
Nonlinear optical (NLO) microscopies (two photon microscopy, TPM or second harmonic imaging microscopy, SHIM) received a tremendous interest from biologists because their confocal character allows non invasive 3D imaging of biological tissues.[1,2] TPM and SHIM are based on fundamentally different NLO processes, two-photon excited fluorescence (TPEF) and second harmonic generation (SHG), respectively. Therefore, they provide complementary information and can be implemented simultaneously in the same microscope.[3] Whereas TPEF labels give information about molecular distribution, the coherent SHG signal is sensitive to the local non centrosymmetric organisation of the dye molecules and find promising applications for the measurement of membrane potential of the neuronal activity of the brain.[4] The emergence of this research field points out the need for new chemical probes specifically designed for that purpose.

To that end, we have defined two different types of requirements that the probe must fulfill: the *function* and the *form* criteria, *i.e.* the appropriateness of probe properties to the physical experimental setup and to the biological target respectively. The *function* requirements are: (i) optimal TPEF and SHG signals for a laser operating near 800 nm (fs Ti-Saphire Laser), (ii) good sensitivity to the membrane electric field, and (iii) good photostability under laser irradiation to prevent photobleaching. We defined the *form* requirement as: (iv) good hydrosolubility in order to avoid the use of toxic co-solvent, and paradoxically (v) a lipophilic character to present a good affinity for the phospholipidic membrane. None of the existing probes, optimised either for TPM or SHIM, actually fulfil all these requirements.[4] Here, we report the synthesis and photophysical properties of chromophores satisfying all the form criteria, *i.e.* combining both SHG and TPEF as well as potential electrochromic properties.

We chose to design push–pull chromophores in which the donor and the acceptor moities can be further functionalised to answer the form requirements. Therefore we focused on dicarboxamide pyridine based dyes (Fig. 1) for following reasons: (i) the four alkyl chains of the acceptor amide moities can be modified to adjust the lipophilic character, (ii) the dialkylamino donor end-group can be easily functionalised with hydrosolubilising fragments, (iii) the nature of the conjugated bridge can tune the optical properties in order to get optimal TPEF and resonance enhanced SHG signal at the operating wavelength, and finally (iv) only aryl-ethynyl backbones are considered to prevent photoisomerisation and photobleaching.[5]

The synthesis of the target compounds was achieved using a double Sonogashira cross-coupling reaction between a terminal alkyne and an iodo-aryl derivative (Scheme 1). Compound **1** was obtained in 89% yield *via* the coupling of 4-dihexylaminophenylene-ethynylene and 4-iodopyridine-dicarboxamide **6**. Further alkynylation of **6** followed by deprotection of the trimethylsilyl moieties led to the formation of the terminal alkyne **8** in 85% yield. The latter compound was involved in a second Sonogashira reaction with 2-iodo-7-dihexylamino-9,9-dihexylfluorene to give **2** in 53% yield after column chromatography.

The absorption spectra in chloroform of **1** and **2** (Fig. 2 and Table 1) reveal a broad band centered at 384 and 411 nm respectively. In addition, both compounds exhibit a yellow–green emission with an intense structureless band at 467 and 525 nm for **1** and **2** respectively.† It is important to note that replacement of a phenyl (**1**) by a fluorenyl (**2**) results in a classical red-shift of the absorption and emission bands but induced a dramatic increase of the quantum yield efficiency from 0.13 to 0.89 (Table 1). In order to assign the transition, density functional theory calculations (B3LYP functional, 6–31G* basis set, Gaussian 03 software) were performed on the model compound



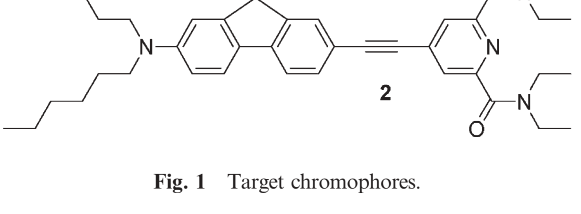


Fig. 1 Target chromophores.

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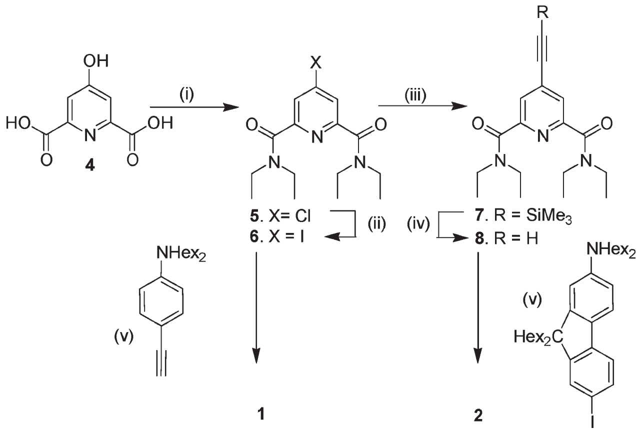
† Electronic supplementary information (ESI) available: Theoretical simulation for **2′**, characterisation of **1** and **2**, absorption and emission spectra in various solvents. See DOI: 10.1039/b610557b



Scheme 1 (i) $SOCl_2$, DMF (cat.), reflux, followed by $HNEt_2$, CH_2Cl_2, reflux, 72%; (ii) HI (aq), H_3PO_3 (cat.), 80 °C, 3.5 h, 62%; (iii) TMS–CCH, $PdCl_2(PPh_3)_2$, CuI, THF–NEt_3, 40 °C, 91% (iv) K_2CO_3, MeOH–CH_2Cl_2, 94%; (v) $PdCl_2(PPh_3)_2$, CuI, THF–NEt_3, 40 °C, 89% (**1**), 53% (**2**).

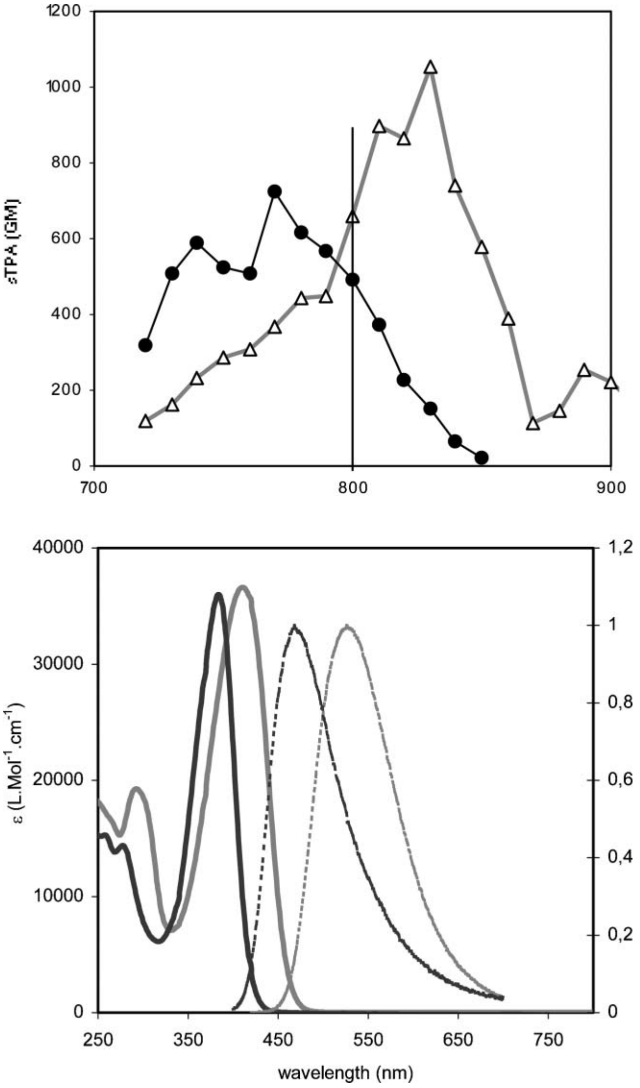


Fig. 2 Absorption and normalised emission (dashed lines) (down) and two-photon excitation (up) spectra of **1** (black) and **2** (gray) in chloroform.

Table 1 Linear and nonlinear optical data

Compounds	1	2
λ_{max}/nm[a]	384	411
ε/L Mol^{-1} cm^{-1}[a]	36000	37000
λ_{em}/nm[b]	467	525
Φ_L[b]	0.13	0.89
$\mu\beta$ ($\mu\beta_0$)/10^{-48} esu[d]	220 (177)	320 (249)
$\lambda^{(2)}_{max}$/nm[e]	770	830
σ_{max}/GM[f]	780	1146
$\sigma_{(800)}\Phi_L$/GPM	69	640

[a] Maximum absorption wavelength in $CHCl_3$. [b] Maximum emission wavelength in $CHCl_3$, quinine sulfate as standard. [d] Measured by EFISH at 1.91 μm in $CHCl_3$, β_0 is deduced from the two-levels model. [e] λ_{max} of the two-photon excitation spectrum. [f] Peak two-photon cross section 1 GM = 10^{-50} cm^4 s per photon.

dimethylaminofluorenylethynyl-pyridine-2,6-dimethyl-carboxamide **2′**. The optimized geometry† has a completely planar structure and TD-DFT calculations indicate a lowest energy transition centered at 422 nm in excellent agreement with the experimental data.† This transition is almost exclusively composed of the HOMO–LUMO electronic excitation from the donor to the acceptor part of the molecule respectively (Fig. 3) and therefore presents a marked charge transfer character.

In addition, both compounds exhibit a positive solvatochromism in absorption and in emission which is consistant with the ICT character of the optical transition. For instance, the emission of **1** is shifted from 408 nm in pentane to 525 nm in acetone. Fig. 4

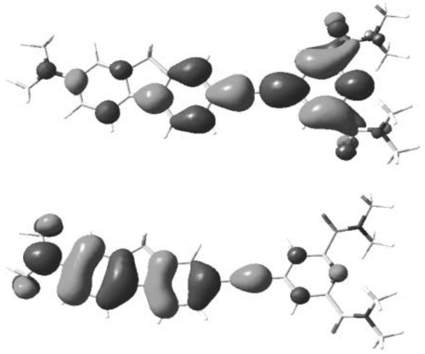


Fig. 3 HOMO (bottom) and LUMO (top) of **2′** from DFT calculations.

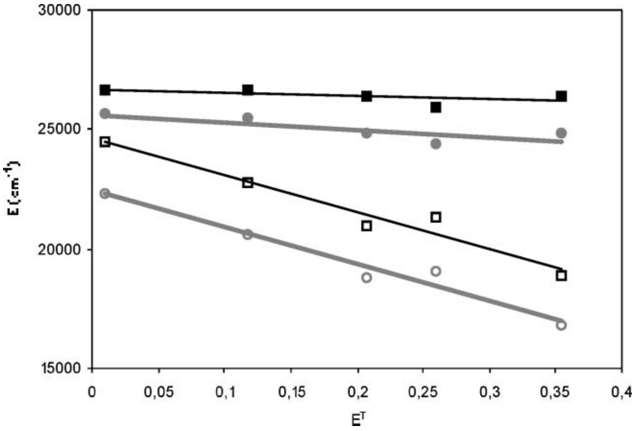


Fig. 4 Solvatochromic plot in absorption (filled symbol) and in emission (open symbol) for **1** (●) and **2** (■) respectively.

shows the linear correlation between absorption and emission energies *versus* the Reichardt's polarity scale parameter[6] E^T. Interestingly, **1** and **2** present a small solvatochromism in absorption and a strong one in emission which is characteristic of molecules featuring a small dipole moment in the ground state and a strong one in the excited state. This experimental interpretation is corroborated by the small permanent dipole moment (μ_f = 3.9 D) calculated for the ground state of **2′**. From this solvatochromic study, it is possible to conclude that **1** and **2** present a large $\Delta\mu = \mu_e - \mu_f$ value which is also the key requirement for electrochromism,[7] one of the molecular mechanisms that results in voltage-dependent optical properties.[8]

The second order NLO properties of the dipolar chromophores **1** and **2** have been measured by EFISH using 1.91 μm as the fundamental laser wavelength[9] and the static data were deduced from the two level model (Table 1).[10] As expected, **2**, which has a longer π-conjugated backbone, exhibits a higher $\mu\beta_0$ value than **1**. In the case of **2**, the static hyperpolarisability, β_0(**2**) can be estimated as 64.10^{-30} esu from the calculated permanent dipole moment μ = 3.9 D. It is worth noting that this significant NLO activity is of the same order of magnitude as that of the chromophores previously used for SHIM namely alkylstilbazonium dyes[11] (β_0 about 50.10^{-30} esu) or retinal[12] (β_0 = 114.10^{-30} esu). Finally, the two-photon absorption properties were measured using a femtosecond Ti-Saphire laser. The two photon excitation spectra (Fig. 2) reveal a broad absorption band in the 700–900 nm range. As expected for noncentrosymmetric compounds, the two photon absorption maximum wavelength $\lambda^{(2)}{}_{\max}$, is twice that of the single photon absorption (Table 1). The two-photon cross sections $\sigma_{\max}$ are important 780 and 1146 GM for **1** and **2** respectively. These values are closed to that of the best chromophores used for TPM application ($\sigma_{\max} \sim 10^3$ GM),[13] and far higher than that of endogenous or commercial dyes ($\sigma_{\max}$ in the range 1–10^2 GM).[14] Interestingly, the effective two photon absorption cross section at the operating wavelength of the experimental microscopy setup, $\sigma_{(800)}\Phi_L$ remains very high for **2** (640 GM) whereas it is dramatically smaller in the case of **1** (69 GM) which is of prime importance for practical utilisation. This marked difference is due to the presence of the fluorenyl moities that ensure a very high quantum yield efficency.

In conclusion, this study describes the design of chromophores optimized for both SHG and TPEF, exibiting potential electrochromic behavior. It is worth noting that quadrupolar chromophore optimized for TPEF are centrosymetric hence not active in SHG. On the other hand, dipolar chromophores already used for second harmonic imaging microscopy exhibit a very moderate two-photon cross-section ($\sigma_{\max} < 10$ GM).[15] Most importantly, compound **2** having the highest effective two-photon cross-section at the operating wavelength fulfils all the *function* requirements described in introduction and should be a very good model for the design of an efficent membrane potential probe. Studies are currently in progress to adapt this chromophore to the biological medium and fulfil the *form* requirements, amphipilic character and hydrosolubility. To that end, donor and acceptor moieties will be functionalised by hydrosolubilizing groups and alkyl chains, respectively. Additional functionalisation on the fluorene ring will allow the control of the hydrophilic–hydrophobic balance.

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