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COMMUNICATION

Influence of bromine substitution pattern on the singlet oxygen generation efficiency of two-photon absorbing chromophores†

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A molecular engineering strategy based on rational variations of the bromine substitution pattern in two-photon absorbing singlet oxygen sensitizers allows studying the relations that exist between the positioning of an inter-system crossing promoter on the charge-transfer chromophore and its ability to generate singlet oxygen.

Photodynamic therapy (PDT) is a therapeutic protocol which is currently limited to the treatment of several surface diseases, like skin or mucosal cancers.[1–3] It requires a photosensitizer,[4,5] *i.e.* a molecule possessing an accessible triplet excited state able to generate singlet oxygen ($^1O_2$) by an energy transfer to ground state oxygen, ultimately leading to cell death.[6–8] Two-photon sensitized PDT is an emerging approach, which allows a deeper penetration of light into tissues (since irradiation operates in the biological transparency window, 700–1100 nm) and a better control on the irradiation volume than its one-photon counterpart.[9–18] Thus, it is possible to envision more in-depth treatment of tumors, while minimizing detrimental side-effects on the neighboring healthy tissues. Ideal two-photon absorbing (TPA) chromophores for PDT must possess two essential spectroscopic characteristics: (i) a large two-photon absorption cross section (σ_{TPA}) at the excitation wavelength and (ii) a high quantum yield of singlet oxygen generation (ϕ_Δ), which is relevant to the efficiency of singlet-to-triplet intersystem crossing (ISC) of the chromophore in its excited state.[19]

These two parameters can be used to define a "figure of merit" at 800 nm‡ ($\sigma_{\mathrm{TPA800}} \times \phi_\Delta$) for the photosensitizer.[12] Two-photon absorbing chromophores have been the subject of various studies during the last two decades, and the associated rules of molecular engineering and the structure–property relationship are now well established.[20–23] In particular, it has been established that chromophores that feature electronic transition of the internal charge-transfer (ICT) type are optimal candidates for TPA, especially those with quadrupolar symmetry.[7] Comparatively, in spite of the fact that many studies have been focused on improving ISC efficiency and ultimately one-photon PDT activity in chromophores with localized excited states (porphyrin[24] and rhodamine[25] derivatives, mostly) only a few authors have systematically studied the relationship that exists between structural parameters and $^1O_2$ generation efficiency in the context of ICT chromophores. In most cases, $^1O_2$ generation is achieved through substitution of the chromophore with a group responsible for the ISC. This approach, which we have also recently explored,[26] has been successful in designing efficient chromophores for two-photon PDT;[13–15] yet, the relations between ISC efficiency (that is, the mechanism by which the delocalized ICT excited singlet state evolves into a localized triplet state) and the position and/or number of ISC promoters remain poorly understood in the context of ICT chromophores. With the aim to more particularly address that point, we initiated a systematic study based on an original engineering approach: it markedly differs from previously published works on the topic, since the chemical nature of the ISC promoter (two bromine substituents in the 2,5-positions on a phenyl moiety) is maintained in all molecules, and the variations we operate concern exclusively its number and positioning along the ICT axis ("p + 1" and/or "p + 2" position, Fig. 1). This allows us to vary exclusively the ISC kinetic constant (k_{ISC}) while keeping all other kinetic constants almost unaffected (*vide infra*). In this preliminary study, we show that this systematic approach can be used to tune the $^1O_2$ quantum yield generation along with other spectroscopic features of interest, to a rather unexpected extent.

Following the aforementioned strategy, and using previously reported compound **1**[15,18] (Fig. 1) as a benchmark and model, a series of four quadrupolar chromophores was designed and synthesized. For convenience, those chromophores will be referred to as **0Br**, **2Br**, **4Br** and **6Br** in the following. The series was obtained through a convergent "building-blocks" synthesis,

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† Electronic supplementary information (ESI) available: Synthesis, analytical characterization, discussion on the excited state kinetics, ^1H and ^{13}C NMR spectra, HPLC traces, solvatochromism study, 77 K time-gated luminescence, fluorescence and $^1O_2$ phosphorescence plots, including those of compound **2Br′**. See DOI: 10.1039/c2ob25536g

based on successive Sonogashira–Hagihara reactions from the electron donating group to the molecule's core. For experimental details and characterizations, see ESI.†

Absorption and emission spectra were obtained using diluted $CHCl_3$ solutions of the compounds at 298 K (Fig. 2 and Table 1). In all cases, a broad low-energy absorption band is observed, centered at 389, 395, 410 and 435 nm, for **0Br**, **2Br**, **4Br** and **6Br**, respectively. As expected, this band can be unambiguously ascribed to an ICT transition, regarding its characteristic shape, large Stokes shift and positive solvatochromism (Fig. S4†). The bathochromic shift along the series is in agreement with the electro-deficiency induced by the increased number of dibromophenyl moieties along the conjugated skeleton. All compounds are luminescent under irradiation in their ICT band, with a broad, solvatochromic ICT emission band centered at 476, 490, 500 and 560 nm, for **0Br**, **2Br**, **4Br** and **6Br** in chloroform, respectively. The decay times (Table 1, Fig. 4) are in the ns range, which indicates a fluorescence emission process.

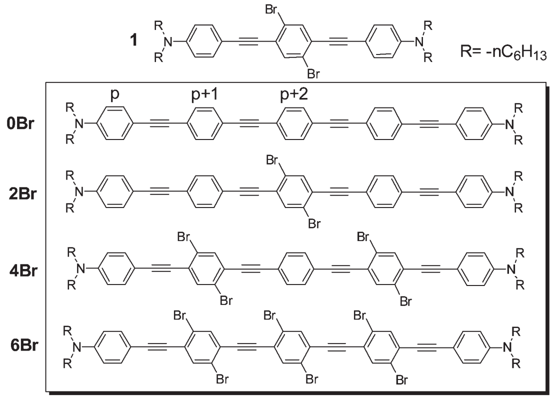

Fig. 1 Compounds of the study and benchmark **1** [ref. 15 and 18]. p + *n* (*n* = 1, 2) refers to the phenyl ring position relative to the terminal aniline.

Attempts to observe triplet excited state emission of the compounds at room temperature have to date remained unsuccessful. However, at low temperature in glassy solution, structured phosphorescence emissions were observed, except for **0Br** (Table 1, Fig. S5†).

$^1O_2$ generation quantum yield (ϕ_Δ) quantifies the chromophore efficiency as a potential PDT sensitizer. It was determined from the characteristic $\Delta^1O_2 \rightarrow \Sigma^3O_2$ phosphorescence at 1270 nm. All brominated compounds (**2Br**, **4Br** and **6Br**) generate singlet-oxygen, with quantum yields of 0.10, 0.50 and 0.35, respectively (Table 1, Fig. S6†). Interestingly, fluorescence quantum yields and lifetimes evolve opposite to (and almost mirror) the $^1O_2$ generation quantum yield (Fig. 3). This tendency is in excellent agreement with the fact that $^1O_2$ quantum yield can be directly

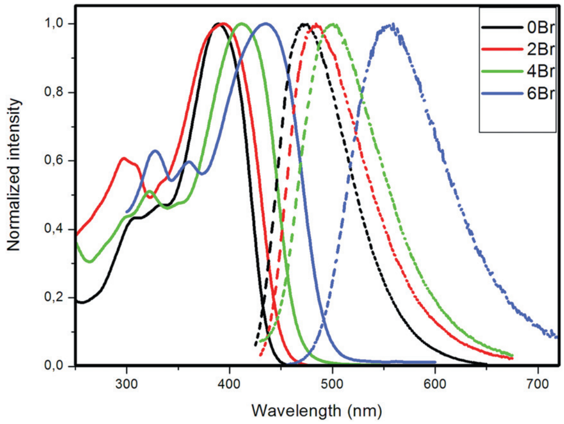

Fig. 2 Absorption (full) and emission (dash) spectra ($CHCl_3$, 298 K).

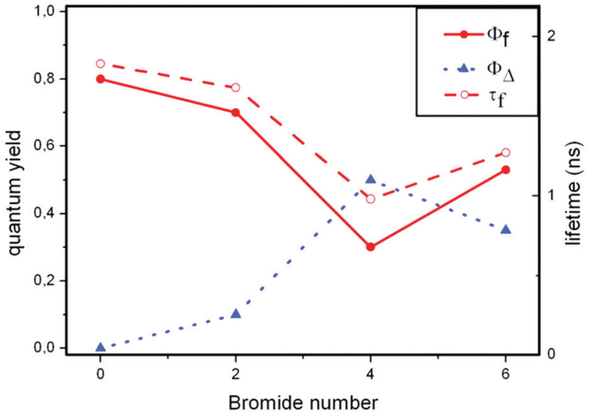

Fig. 3 Evolution of the photophysical parameters (ϕ_f, ϕ_Δ, τ_f) among the chromophore's series (298 K, $CHCl_3$).

Table 1 Relevant spectroscopic data for the study

	0Br	**2Br**	**4Br**	**6Br**	***1***^{d}
λ_{max} (ε)	389 (79×10^3)	395 (46.6×10^3)	410 (49.9×10^3)	435 (53.8×10^3)	*421 (91.5×10^3)*
λ_{fluo} (τ_f)	476 (1.83)	490 (1.68)	500 (0.96)	560 (1.27)	*458 (0.26)*
ϕ_f ^{a}	0.80	0.70	0.30	0.53	*0.25*
λ_{phos} ^{b}	—	530, 569	594, 637	600, 674	*586*
ϕ_Δ ^{c}	—	0.10	0.50	0.35	*0.53*
$\lambda_{TPA\ max}$ (σ_{TPA})	750 (460)	760 (450)	830 (380)	880 (260)	*550 (730)*
$\sigma_{TPA800} \times \phi_\Delta$	0	30	175	40	*37*

λ in nm, ε in L mol^{-1} cm^{-1}, τ_{fluo} in ns and σ_{TPA} in Goeppert-Mayer (GM). λ_{exc} were 390 nm, 395 nm, 410 nm and 435 nm. ^{a} Coumarin 153 in MeOH as a standard, $\phi_{ref} = 0.45$. ^{b} Emission at 77 K (MeOH–EtOH, 1 : 4, v/v, time delay of 65 μs). ^{c} Naphthalenone in $CHCl_3$ as a standard, $\phi_\Delta = 1$. ^{d} From ref. 18.

connected to ISC efficiency, since all kinetic constants other than k_{ISC} are shown to be unchanged as a result of the structural similarity of all chromophores within the series (see ESI† for a full demonstration). **4Br** exhibits a $^1O_2$ quantum yield (ϕ_Δ = 0.50) that is five times higher than its dibrominated analogue **2Br** (ϕ_Δ = 0.10) with a concomitant drop in both fluorescence quantum yields and lifetime values (Fig. 3, Table 1). This trend could potentially be ascribed either to a synergistic enhancement of the ISC efficiency related to the increased number of bromines on the chromophore, or to a positional effect, with lateral ("p + 1") substitution providing higher ISC efficiency than the central ("p + 2") one in this particular series. Examination of the results obtained with **6Br** clearly rules out the first hypothesis: indeed, in spite of the increased number of heavy atom substituents, **6Br** displays a loss of $^1O_2$ generation efficiency (ϕ_Δ = 0.35) and a concomitant increase of the fluorescent quantum yield and lifetime compared to **4Br**, which altogether can be ascribed to a lower ISC efficiency. To make these comparisons more conclusive, an additional compound bearing two bromines on one "p + 1" position only (referred to as **2Br′**, ESI†) was synthesized and its spectroscopic properties were investigated. Because of the significantly less modular and straightforward synthetic approach required for the obtention of the latter, and also because of complicated symmetry issues that would extend the discussion on its nonlinear properties out of the scope of this communication, this compound will not be extensively featured in the present article. Its synthesis and detailed properties will be reported elsewhere in the near future, its structure and main photophysical data are given as ESI.† From the aforementioned spectroscopic study, it was possible to establish that **2Br′** had fluorescence (ϕ_f = 0.32) and singlet oxygen quantum efficiencies (ϕ_Δ = 0.50), but also fluorescence lifetime (τ_f = 0.93 ns), that were, within experimental error, undistinguishable from those of **4Br**.

These results constitute unambiguous evidence that, rather than the number of $^1O_2$ generating moieties, their detailed position on the carbon backbone is the determining parameter that drives ISC efficiency within our series: an ideal positioning of the heavy atoms seems to be on the peripheral phenyls next to the aniline ring ("p + 1" position, as in **4Br** and **2Br′**), whereas bromine substitution on the "p + 2" (**2Br**) ring provides in comparison a much lower efficiency. For a given position, the number of bromine substituted phenyls does not seem to play a significant role (if any) in the chromophore efficiency (as seen by the similarity for the results obtained with **4Br** and **2Br′**). Interestingly, and quite unexpectedly, the presence of bromine atoms in both "p + 1" and "p + 2" positions has a detrimental effect on the ISC, as seen when comparing ϕ_Δ of **4Br** and **6Br** (Fig. 3).

Finally, TPA cross sections were determined by two-photon induced fluorescence measurements on the quadrupolar chromophore series (ESI†). In the biological transparency window (750–900 nm), all compounds show significant TPA cross sections, with values ranging from 250 GM (**6Br**) to 460 GM (**0Br**) (Fig. 4, Table 1). As for their linear absorption spectra, a significant bathochromic shift is observed throughout the series. **0Br** and **2Br** give the best results in absolute values, but their absorption maxima are slightly below the optimal transparency window. **6Br** shows a maximal TPA in the NIR but with a moderate cross section value (<200 GM), whereas **4Br** shows a broad TPA peak, with a cross section larger than 300 GM from 770 to 860 nm. This shows that, besides having a strong influence on $^1O_2$ generation, the bromine substitution pattern can be used to tune TPA properties ($\lambda^{TPA}{}_{max}$) of the chromophore. Taken together, this leads **4Br** to display the best figure of merit in the biological window ($\sigma_{TPA800} \times \phi_\Delta$, Table 1) of all quadrupolar compounds in the series (including **1**), and makes it a relevant structure for future PDT applications, provided that some modifications are introduced to render it biocompatible, as previously illustrated with related chromophore **1**.[18]

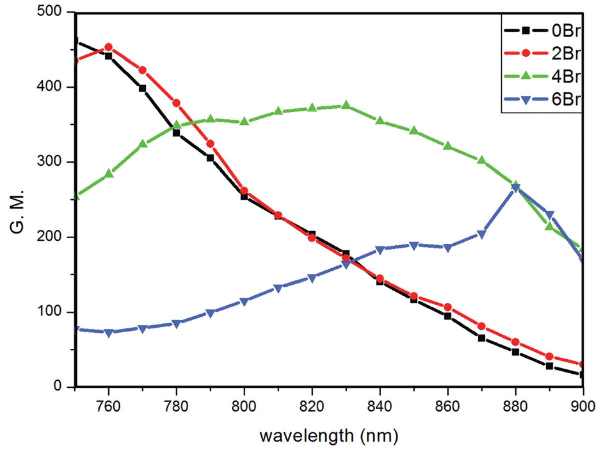

Fig. 4 Two-photon absorption spectra (CH_2Cl_2, 298 K).

In summary, this study allowed us, owing to an original and straightforward molecular engineering approach, to progress significantly in our understanding of some of the relations that exist, in ICT chromophores, between the position and number of a given ISC promoter (2,5-dibromophenyl) and the $^1O_2$ generation efficiency. This is of particular significance in the context of molecular engineering for two-photon PDT applications, since the position of the heavy atom has only scarcely been discussed in previous published works relying on a similar "heavy atom" strategy for $^1O_2$ sensitization in ICT chromophores,[27,28] and never investigated in such a systematic way. Besides, we have also shown that varying the bromine substitution pattern could be used to tune the two-photon absorption properties of the molecule, as a result of the electron withdrawing effect of the bromine atoms. Altogether, the results of this study suggest that, for works dealing with ISC improvement in ICT chromophores, caution should be taken not only with the choice of the ISC generating moiety, but also with its number and – most importantly – positioning along the chromophore ICT transition axis. Theoretical and experimental investigations are underway to gain better understanding on the structure-to-property relationships in this class of brominated two-photon photosensitizer.

Notes and references

‡ 800 nm is taken as a reference, since it is the most classically used wavelength for *in vivo* applications based on Ti-sapphire LASER.

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