

# New quadrupolar fluorophores with high two-photon excited fluorescence

Lionel Ventelon,<sup>a</sup> Laurent Moreaux,<sup>b</sup> Jerome Mertz<sup>b</sup> and Mireille Blanchard-Desce\*<sup>a</sup>

<sup>a</sup> Département de Chimie, Ecole Normale Supérieure (UMR 8640), 24, rue Lhomond, 75231 Paris Cedex 05, France. E-mail: mbdesce@junie.ens.fr

<sup>b</sup> Laboratoire de Physiologie, Neurobiologie et Diversité Cellulaire (UMR 7637), Ecole Supérieure de Physique et Chimie Industrielle, 10, rue Vauquelin, 75231 Paris Cedex 5, France

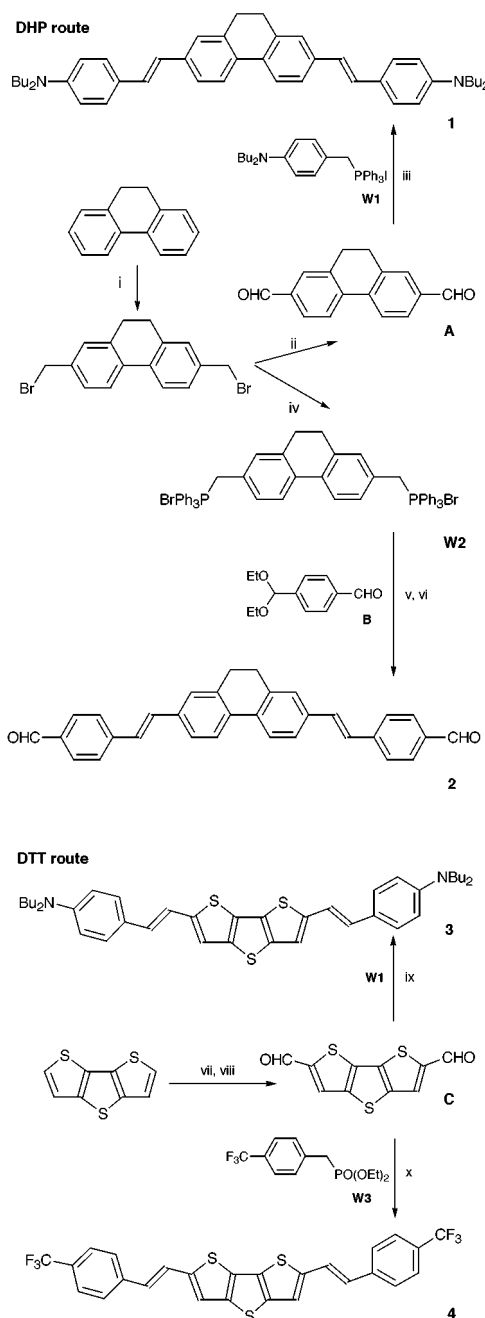
Received (in Cambridge, UK) 30th July 1999, Accepted 8th September 1999

New quadrupolar fluorophores that exhibit enhanced two-photon excited fluorescence were designed according to a molecular engineering approach based on push-push and pull-pull elongated molecules built from a rigid conjugated core (either dihydrophenanthrene or dithienothiophene).

Molecular two-photon absorption (TPA)<sup>1</sup> has found many applications over recent years, including optical power limitation,<sup>2</sup> three-dimensional optical data storage,<sup>3</sup> microfabrication,<sup>4</sup> up-conversion,<sup>5</sup> *etc.* Applications that specifically require excited state fluorescence include two-photon excited fluorescence (TPEF) microscopy,<sup>6</sup> which has gained widespread popularity in the biology community owing to its ability to image at an increased penetration depth in tissue with reduced photodamage. Key ingredients for efficient molecular TPEF are a large two-photon absorption cross section  $\sigma_2$ , and a high fluorescence quantum yield  $\Phi$ . In most cases, TPEF microscopy applications have relied on molecules originally designed for one-photon fluorescence.<sup>7</sup> It has become increasingly clear, however, that molecules specifically engineered for TPEF may significantly outperform these more conventional molecules.<sup>8</sup> To this end, we have designed, synthesised and characterised new quadrupolar molecules which exhibit enhanced TPEF.

It has been recently observed<sup>8a</sup> that symmetrical phenylene-vinylene oligomers bearing electron-releasing (or electron-withdrawing) groups can display very large  $\sigma_2$ , seemingly correlated to a symmetrical quadrupolar charge redistribution occurring between the edges and the centre of the molecule. Following some of these observations, our goal was to achieve significant two-photon absorption while maintaining high fluorescence quantum yields by the symmetrical grafting of two semi-rigid conjugated rods bearing either donor or acceptor terminal substituents onto a rigid conjugated central building block. The central conjugated block is designed to allow electronic conjugation and high fluorescence yield, and may assist quadrupolar charge redistribution. The dihydrophenanthrene (DHP) and dithienothiophene (DTT) systems, which permit electronic delocalisation and possess decreased rotational degree of freedoms (due to the fused rings in the case of DTT and to the presence of the bis(methylene) bridge in the case of DHP), appeared to be appropriate central moieties. Styrene moieties were selected as conjugated rods.

Based on the above strategy, we have prepared molecules **1-4** that possess an extended conjugated system<sup>9</sup> (approximately 22–24 Å long) and either donor (push-push molecules **1, 3**) or acceptor (pull-pull molecules **2, 4**) terminal groups (Scheme 1). Molecules derived from DHP can be prepared *via* a double Wittig condensation from symmetrical bisfunctional reagents derived from DHP. Wittig reaction under liquid-liquid<sup>10</sup> or solid-liquid phase transfer conditions<sup>11</sup> of 2.1 equiv. of phosphonium salt **W1**<sup>12</sup> with bisaldehyde **A**<sup>13</sup> afforded push-push molecule **1** in 60% yield after purification by column chromatography on silica gel. Conversely, pull-pull molecule **2** was prepared in 90% yield by reaction of bisphosphonium **W2** with 2 equiv. of commercially available aldehyde **B**, followed by acidic deprotection. The key reagent **W2** was obtained from DHP according to a two step procedure in 50% overall yield.



**Scheme 1** Reagents and conditions : i, (CH<sub>2</sub>O)<sub>3</sub>, HBr, H<sub>3</sub>PO<sub>4</sub>, 24 h, 90 °C; ii, NaHCO<sub>3</sub>, DMSO, 4 h, 115 °C; iii, **W1** (2.1 equiv.), CH<sub>2</sub>Cl<sub>2</sub>, 50% NaOH, 5 h, room temp.; iv, P(Ph)<sub>3</sub> (2 equiv.), CH<sub>2</sub>Cl<sub>2</sub>, 24 h, room temp.; v, **B** (2 equiv.) CH<sub>2</sub>Cl<sub>2</sub>, 50% NaOH, 24 h, room temp.; vi, 10% HCl, THF, 1 h, room temp.; vii, BuLi, THF, -78 °C; viii, DMF, -78 °C; ix, **W1** (2.1 equiv.), KOBu<sup>t</sup>, CH<sub>2</sub>Cl<sub>2</sub>, 5 h, room temp.; x, **W3** (2.2 equiv.), NaH, THF, 24 h, room temp.

**Table 1** One and two-photon absorption and fluorescence properties of fluorophores **1–4**. The experimental uncertainty is estimated to be  $\pm 15\%$  for TPEF measurements and  $\pm 5\%$  for fluorescence quantum yield determinations.

	$\lambda_{\max}(\text{abs})/\text{nm}$	$\lambda_{\max}(\text{em})/\text{nm}$	$\Phi$	$\lambda_{\max}(\text{TPA})/\text{nm}$	$\sigma_2\Phi/10^{-50} \text{ cm}^4 \text{ s photon}^{-1}$	$\sigma_2/10^{-50} \text{ cm}^4 \text{ s photon}^{-1}$
<b>1</b>	421	540	0.77	765	930	1200
<b>2</b>	397	513	0.83	< 730	> 260 <sup>a</sup>	> 320
<b>3</b>	465	582	0.63	795	340	530
<b>4</b>	424	510	0.44	< 730	> 80 <sup>a</sup>	> 180

<sup>a</sup>  $\sigma_2\Phi$  values measured at 730 nm, the TPA maximum being blue-shifted in the case of fluorophores **2** and **4**.

The same methodology was implemented for the synthesis of molecules **3** and **4** derived from DTT which were prepared in 80–90% yield by reacting bisaldehyde **C**<sup>14</sup> under solid-liquid phase transfer conditions<sup>11</sup> with 2.2 equiv. of either phosphonium salt **W1** or phosphonate **W3**. Molecules **1–4** were isolated as pure all-*E* compounds after crystallisation as established by NMR, elemental analyses and/or high resolution mass spectrometry.

The two-photon excitation cross sections were determined by comparing the TPEF of molecules **1–4** to that of fluorescein using a mode-locked Ti:sapphire laser operating between 730 and 970 nm and delivering linearly-polarised  $\sim 80$  fs pulses at 80 MHz. This experimental protocol is described in detail by Xu and Webb<sup>7</sup> and provides the TPEF cross section  $\sigma_2\Phi$ . The corresponding  $\sigma_2$  values can then be derived by determining the fluorescence quantum yield  $\Phi$  from fluorescence measurements.

The experimental data are collected in Table 1. We conclude from these data that fluorophores **1–4** indeed combine very large two-photon absorption cross-sections and high fluorescence quantum yields. In particular fluorophores **1** and **3** display  $\sigma_2$  values more than two orders of magnitude larger than *p*-bis[*o*-methylstyryl]benzene,<sup>7</sup> a shorter generic molecule that lacks donor or acceptor end groups. This demonstrates the importance of the donor (or acceptor) end groups and of the length and electronic function of the central moiety, both of which govern quadrupolar charge redistribution. We note that fluorophores **1** and **3** exhibit very large TPEF cross sections over a wide spectral range (Fig. 1) spanning the visible red to the near IR (700–970 nm). This spectral window is particularly advantageous for imaging in biological tissues.<sup>15</sup> Both fluorophores **1** and **3** have a TPEF cross section about an order of magnitude larger than conventional fluorophores such as fluorescein or rhodamine. Push-push fluorophores **1** and **3** appear to lead to larger TPA cross-sections than corresponding pull-pull deriva-

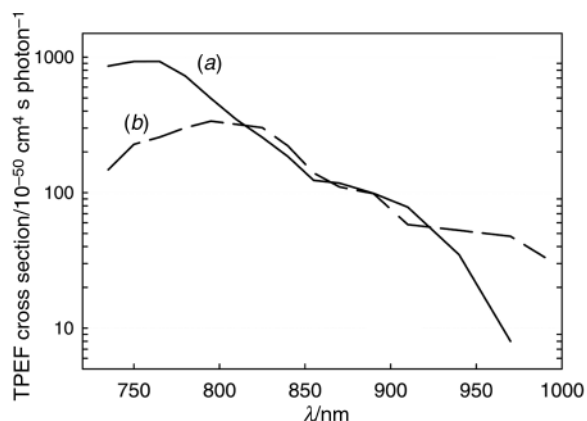
tives **2** and **4**, concomitant with a red-shift of both one-photon absorption and TPA maxima. Fluorophores **2** and **4** which maintain large TPA cross-sections and display excellent transparency in a wide range of the visible region are promising candidates for optical power limitation applications.

In summary, we have demonstrated a successful strategy for the design of fluorophores that combines very large two-photon absorption cross sections and high fluorescence quantum yields. This opens interesting prospects for several applications, particularly in the fields of two-photon microscopy and optical power limitation.

We thank the Délégation Générale pour l'Armement (DGA) for a fellowship to L.V. and the CNRS for a fellowship (BDI) to L.M.. We also acknowledge the Institut Curie for financial support.

## Notes and references

- M. Göppert-Mayer, *Ann. Phys. (Leipzig)*, 1931, **9**, 273; W. L. Petitcolas, *Annu. Rev. Phys. Chem.*, 1967, **18**, 233.
- G. S. He, G. C. Xu, P. N. Prasad, B. A. Reinhardt, J. C. Bhatt and A. G. Dillard, *Opt. Lett.*, 1995, **20**, 435; J. E. Ehrlich, X. L. Wu, I.-Y. S. Lee, Z.-Y. Hu, H. Röckel, S. R. Marder and J. W. Perry, *Opt. Lett.*, 1997, **22**, 1843.
- D. A. Parthenopoulos and P. M. Rentzepis, *Science*, 1989, **245**, 843; J. H. Strickler and W. W. Webb, *Opt. Lett.*, 1991, **16**, 1780.
- B. H. Cumpston, S. P. Ananthavel, S. Barlow, D. L. Dyer, J. E. Ehrlich, L. L. Erskine, A. A. Heikal, S. M. Kuebler, I.-Y.S. Lee, D. McCord-Maughon, J. Qin, H. Röckel, M. Rumi, X.-L. Wu, S. R. Marder and J. W. Perry, *Nature*, 1999, **398**, 51.
- A. Mukherjee, *Appl. Phys. Lett.*, 1993, **62**, 3423; J. D. Bhawalkar, G. S. He, C.-K. Park, C. F. Zhao, G. Ruland and P. N. Prasad, *Opt. Commun.*, 1996, **124**, 33.
- W. Denk, J. H. Strickler and W. W. Webb, *Science*, 1990, **248**, 73.
- C. Xu and W. W. Webb, *J. Opt. Soc. Am. B*, 1996, **13**, 481.
- (a) M. Albota, D. Beljonne, J.-L. Brédas, J. E. Ehrlich, J.-Y. Fu, A. A. Haeikal, S. E. Hess, T. Kogej, M. D. Levin, S. R. Marder, D. McCord-Maughon, J. W. Perry, H. Röckel, M. Rumi, G. Subramaniam, W. W. Webb, X.-L. Wu and C. Xu, *Science*, 1998, **281**, 1653; (b) B. A. Reinhardt, L. L. Brott, S. J. Clarson, A. G. Dillard, J. C. Bhatt, R. Kannan, L. Yuan, G. S. He and P. N. Prasad, *Chem. Mater.*, 1998, **10**, 1863.
- Increasing the extent of quadrupolar charge redistribution is expected to lead to an increase of  $\sigma_2$ .
- M. Blanchard-Desce, I. Ledoux, J.-M. Lehn, J. Malthête and J. Zyss, *J. Chem. Soc., Chem Commun.*, 1988, 737.
- V. Alain, S. Rédoglia, M. Blanchard-Desce, S. Lebus, K. Lukaszuk, R. Wortmann, U. Gubler, C. Bosshard and P. Günter, *Chem. Phys.*, 1999, **245**, 51.
- M. Blanchard-Desce, C. Runser, A. Fort, M. Barzoukas, J.-M. Lehn, V. Bloy and V. Alain, *Chem. Phys.*, 1995, **199**, 253.
- A. Helms, D. Heiler and G. McLendon, *J. Am. Chem. Soc.*, 1992, **114**, 6227.
- O.-K. Kim, A. Fort, M. Barzoukas, M. Blanchard-Desce and J.-M. Lehn, *J. Mater. Chem.*, 1999, **9**, 2227.
- C. Xu, W. Zipfel, J. B. Shear, R. M. Williams and W. W. Webb, *Proc. Natl. Acad. Sci., U.S.A.*, 1996, **93**, 10763.



**Fig. 1** TPEF excitation spectra of fluorophores (a) **1** and (b) **3** dissolved in DMSO ( $10^{-4}$  M).

Communication 9/06182G