

A modular approach to two-photon absorbing organic nanodots: brilliant dendrimers as an alternative to semiconductor quantum dots?†

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Received (in Cambridge, UK) 6th December 2005, Accepted 20th December 2005

First published as an Advance Article on the web 19th January 2006

DOI: 10.1039/b517270e

Nanoscope fluorescent dendrimers having up to 96 two-photon chromophores and showing very large two-photon absorption cross-sections (up to 56 000 GM) were designed as a complementary “organic” alternative to quantum dots.

For more than a decade, two-photon absorption (TPA) has attracted increasing attention in relation with various applications, such as 3-D microfabrication,¹ optical data storage,² photodynamic therapy,³ and optical power limiting.⁴ Two-photon-excited fluorescence (TPEF) has been found to be of particular interest for the biological community: two-photon laser scanning fluorescence microscopy offers the advantages of imaging deeper in living tissues (down to 500 μm), with reduced photodamage and background fluorescence and with 3-D spatial resolution.⁵ In addition to biological imaging, TPEF can also be of interest for 3-D imaging in materials. These applications call for the design of new luminophores whose TPA cross-sections are optimised in the spectral range of interest: 700–1200 nm corresponding to the combination of reduced scattering and minimal absorption in (bio)organic materials. Recently semiconductor nanocrystals (quantum dots: QDs) have been shown to provide a particularly effective approach to fluorescent labels with extremely large TPEF cross-sections (up to 47 000 GM).⁶ Indeed these brilliant nanoobjects (typically of 3 nm radius without the encapsulating polymers) have been heralded as useful materials for biological imaging and have gained overwhelming popularity.^{7,8} However, these inorganic systems (such as those made from zinc sulfides and cadmium selenides⁶) suffer from several drawbacks including toxicity and blinking.

In this paper we demonstrate that an “all organic” alternative approach can lead to nanoobjects with similar geometry and size as semiconductor QDs showing competitive TPEF performance. Organic “nanodots” are built from the grafting of a discrete and large number of optimised two-photon (TP) chromophores on the periphery of dendrimers. In addition this “organic nanodots” strategy offers a modular route for (i) molecular control of

photophysical properties, (ii) modulation of solubility in various environments (*via* addition of external solubilising groups) and (iii) further covalent functionalisation for additional functionality.

Recent work has shown that conjugated dendrimers based upon the assembly of TP chromophores in dendrimeric architectures⁹ exhibit very large TPA cross-sections (up to 11 000 GM). Pioneering work by Prasad, Fréchet and co-workers shows that covalent assembling of TP chromophores within a more flexible dendrimeric assembly leads to high TPA cross-sections (up to 2600 GM for 8 chromophores†).¹⁰ Such dendritic systems can be derivatised by using an acceptor core leading to interesting systems based on Förster resonance energy transfer for two-photon induced photoluminescence¹¹ or even singlet oxygen formation.¹² Aiming at the design of an “all-organic” object of similar size to inorganic QDs with similar TPEF cross-sections, we have designed dendritic systems bearing up to 96 strongly TPA-active fluorophores (Fig. 1). These fluorophores were quasi-1D quadrupolar systems built from the grafting of donor moieties *via* conjugated spacers onto a fluorenyl core. Such systems are known to exhibit large TPA cross-sections in the spectral range of interest for bioimaging.¹³ The triple bond was chosen as a connector to allow better photostability as compared to the double bond thanks to the absence of photoisomerisation.^{13c}

The “nanodots” were built by grafting an exponentially increasing number of TP-active chromophores **1** on the periphery of phosphorus dendrimers of generation 1 to 4 decorated with terminal P(S)Cl₂ groups.¹⁴ Chromophore **1** was prepared in a three-step sequence starting from commercially available aniline **2a**, involving iodination, Mitsunobu reaction with hydroquinone and Sonogashira coupling with alkyne **4** (Scheme 1). Nucleophilic substitutions involving P(S)Cl₂ end groups¹⁵ and **1** take place easily leading to dendrimers **G1**, **G2**, **G3** and **G4** bearing respectively 12, 24, 48 and 96 chromophores (Scheme 2).

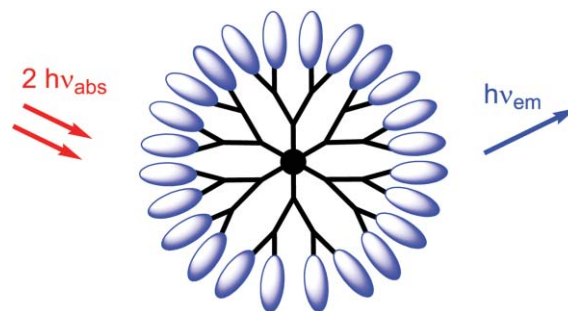


Fig. 1 Schematic representation of dendrimeric two-photon absorbing fluorescent organic “nanodots”.

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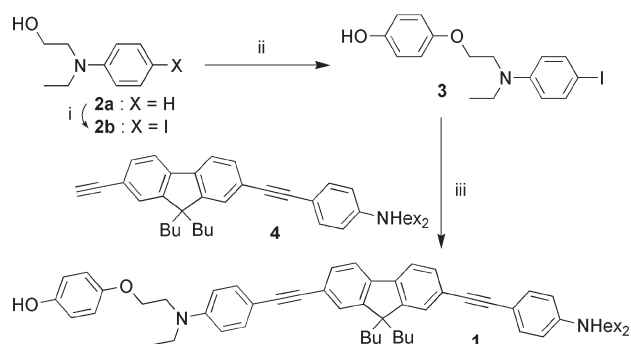
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† Electronic supplementary information (ESI) available: Preparation of chromophore **1** and dendrimers and experimental details of spectroscopic measurements. See DOI: 10.1039/b517270e



Scheme 1 i, I_2 , $NaHCO_3$, CH_2Cl_2 (86%); ii, hydroquinone (3 equiv.), DEAD, PPh_3 , THF, rt, 16 h (51%); iii, **3**, $Pd(PPh_3)_2Cl_2$, CuI, toluene/ Et_3N , 40 °C, 16 h (57%).

Dendrimers **G1–G4** show strong one-photon absorption in the near UV with a nearly linear increase of the extinction coefficient with the number of chromophores (Table 1) as expected from quantitative functionalisation of the dendrimer periphery. As desired, all dendrimers maintain reasonably high quantum yields, even in the highest generation **G4**, which validates our strategy. A clear loss of fine structure and broadening of the emission band (in particular in the red) is observed (Fig. 2). This change in the shape of the emission spectra with increasing generation suggests that interchromophoric interactions occur in the excited state. In contrast, the absorption spectra remain virtually unchanged pointing to the lack of significant interactions in the ground state. A modest decrease in fluorescence quantum yield is also observed with increasing the generation, most probably in relation to interchromophoric interactions in the excited state. The dendrimers **G1–G4** display much lower steady-state fluorescence anisotropies than the parent chromophore **1**. Since rotational depolarization in these bigger molecules is expected to be much

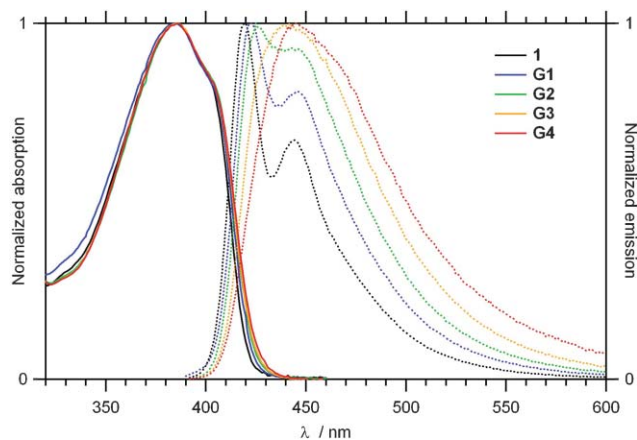
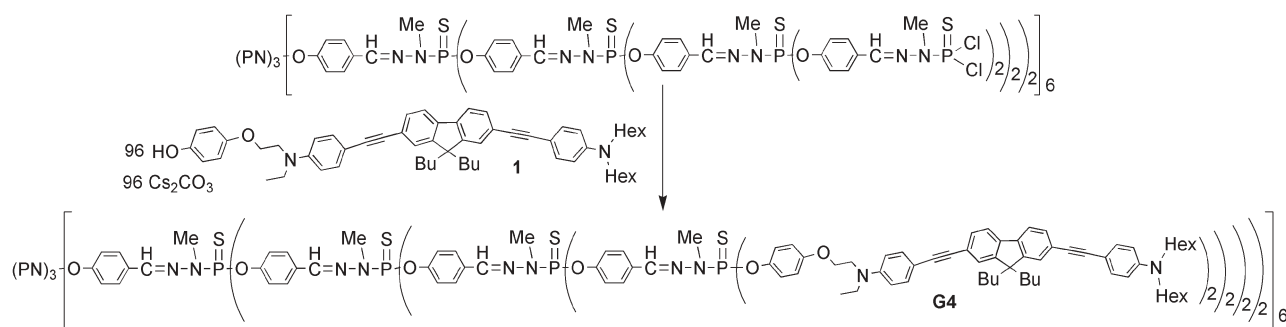


Fig. 2 Normalized absorption and emission spectra in toluene of dendrimers **G1–G4** and model chromophore **1**.

slower, the depolarization must be due to rapid energy transfer between chromophores. Preliminary time-resolved measurements show that this process is much faster than the limited time-resolution of our flashlamp-based TCSPC apparatus (instrumental response 2 ns FWHM). This also explains the fact that we do not observe any fast components in the fluorescence lifetime measurements, only monoexponential decays. Further investigations of the photophysical dynamics are planned.

The TPA of the dendrimers was studied by investigating the TPEF of dendrimers **G1–G4** in toluene. TPEF measurements allow for direct measurement of the TPEF action cross-section $\sigma_2\Phi$, the relevant figure of merit for imaging applications. In addition this method has been recognized as more reliable than nonlinear transmission measurements.¹⁶ The quadratic dependence of the TPEF signal on the excitation intensity was checked for each data point, indicating that no photodegradation or saturation



Scheme 2 Synthesis of the 4th generation dendrimer bearing 96 fluorophore end groups.

Table 1 Photophysical data for model fluorophore **1** and dendrimers **G1–G4** in toluene

	Number of fluorophores	λ_{abs}/nm	$\epsilon/M^{-1} cm^{-1}$	λ_{em}/nm	Φ^a	τ/ns^b	r^c	$\lambda_{TPA(max)}/nm$	σ_2 at $\lambda_{TPA(max)}/GM^d$
1	1	386	84 900	420, 444	0.83	0.67	0.178	702	765
G1	12	385	1 004 000	423, 446	0.75	0.71	0.032	701	8880
G2	24	386	2 035 000	426, 445	0.71	0.69	0.019	701	17 700
G3	48	386	3 785 000	441	0.62	0.71	0.013	701	29 800
G4	96	386	7 101 000	445	0.48	0.66	0.012	705	55 900

^a Fluorescence quantum yield in toluene determined relative to fluorescein in 0.1 N NaOH. ^b Experimental fluorescence lifetime measured by time-correlated single photon counting. ^c Steady-state fluorescence anisotropy. ^d 1 GM = $10^{-50} cm^4 s photon^{-1}$.

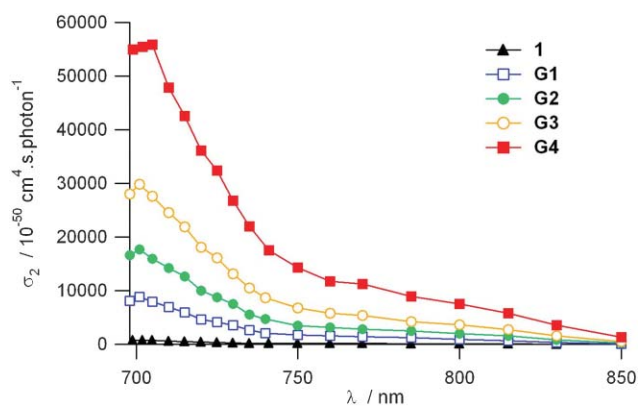


Fig. 3 TPA spectra of dendrimers **G1–G4** and model chromophore **1** determined by femtosecond TPEF measurements in toluene.

occurs. The TPA spectrum of monomeric chromophore **1** was also determined for comparison. As clearly seen from Fig. 3, the TPA cross-section increases linearly with the growing number of chromophores in each generation, leading to very large TPA cross sections, comparable to inorganic QDs. The additive behaviour indicates that the molecular responses of each individual chromophore located on the periphery remain unaffected (as could be expected from the absence of significant interactions in the ground state). In contrast molecular aggregation of individual chromophores (in a solvent promoting aggregation) has been shown to lead to TPA loss.¹⁷ The confinement strategy based on the covalent grafting on a dendritic architecture thus allows avoidance of any deleterious effects on TPA as a result of the aggregation of chromophores in a spontaneous manner. As a result, the maximum value (55 900 GM at 705 nm) obtained for **G4** is comparable to the σ_2 value measured by Webb and co-workers⁶ for the best reported luminescent TP markers, semiconductor quantum dots. It should also be stressed that organic “nanodot” **G4** is about the same size (its radius can be estimated to be about 4 nm¹⁸) as these QDs, demonstrating that the organic route to bright nanoobjects with TPEF cross-sections as high as QDs is viable. In addition, the phosphorous dendrimers have been shown to have very low toxicity,¹⁹ a major advantage for bioimaging applications.

In conclusion, the present results demonstrate that organic “nanodots” represent a promising approach towards fluorescent markers competitive and complementary to semiconductor QDs for TPEF imaging-based applications. In addition the modular route allows access to a variety of organic nanodots with different fluorophores, allowing tuning of the photoluminescence properties. We have validated the proposed strategy with quadrupolar type chromophores that are blue-emitters. Such a route could also be applied to the grafting of quadrupoles that are green-emitters or yellow-emitters and show broader (and even higher) TPA.^{13b} Further functionalisation may also be achieved. In particular adding supplementary layers and decorating the periphery with ammonium groups would yield hydrosoluble dendrimers.²⁰

We gratefully acknowledge financial support from Rennes Métropole (“Allocation d’Installation Scientifique” awarded to MBD and MHVW). JPM thanks the Ministère de la Recherche

(France) for the post-doctoral grant to TRK. We wish to thank Cédric Rouxel for his contribution to synthesis, and Adrien Gilot and Manuel Parent for their help in setting up the TPEF experiment.

Notes and references

‡ TPA data derived from femtosecond measurements.

§ TPEF measurements were performed under excitation with 150 fs pulses from a Ti:sapphire laser, using the protocol of Xu and Webb,²¹ taking into account refractive index effects.²²

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