A V-shaped cationic dye for nonlinear optical bioimaging†

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Received 14th April 2011, Accepted 13th May 2011
DOI: 10.1039/c1cc12163d

A symmetric cationic molecule with D-π-A⁺-π-D architecture was synthesized with high two-photon absorption cross-section (σ2 ≈ 1140 GM). Application as a marker in fluorescence microscopy of living cells revealed its presence inside the cell staining vesicular shape organelles in the cytoplasm. Fluorescence lifetime imaging microscopy shows that it is also able to penetrate within the nucleus.

Among the different avenues that are still challenging and especially promising for future developments in two-photon emission (TPE) of organic dyes are open-shell and charged systems.¹² Cationic and anionic units are exceptional acceptor and donor groups. Cationic dyes are widely used as biomarkers in linear optical imaging. Despite the low nonlinear optical response of the commercially available cationic dyes, there are also many examples of their successful application in TPE microscopy.³⁻⁵ Nevertheless, to the best of our knowledge, only a handful of cationic systems have been targeted for optimization of the nonlinear optical response together with their application in microscopy of living cells. Examples of this are the wide series of cationic styryl cyanine dyes.⁶,⁷ They were reported to have quite low two-photon absorption crosssection (σ2 < 5 GM, 1 GM = 10⁻⁵⁰ cm⁶ s) but they were shown to be efficient staining agents of nucleic-acid-containing organelles in the cytoplasm.⁶,⁷ A series of relatively simple carbazole containing pyridinium dyes have been recently shown to exhibit a very good contrast in staining mitochondria due to their high σ2 (up to 600 GM in acetonitrile).⁸ Charged, water soluble bis(styrylbenzene) derivatives have been prepared with σ2 ≈ 400 GM and an emission quantum yield of 0.2–0.4.⁹,¹⁰ Incubation of HeLa cells with these dyes resulted in bright emission localized in the plasma membrane with no observable internalization.⁹,¹⁰ Other families of cationic molecules have been reported with very good σ2 values but have not been tested yet as staining agents for imaging of in vivo cells. Examples of this are a series of (dimethoxyphenyl)-ethynyl-methylpyridinium based molecules with a linear (A⁻-π-A⁺) structure with σ2 values of up to 3500 GM.¹¹⁻¹³ The drawback is that the emission quantum yields of the best performing systems are relatively low (<0.1).¹² and their solubility in water is limited. Cationic triphenylamine based molecules have been recently reported to have both high emission quantum yields (0.70 in methanol) and high two-photon absorption cross-section (4150 GM).¹⁴ The water solubility issue could be circumvented to some extent by the use of surfactants, with substantial decrease of TPE.

We have synthesized two cationic molecules with a general D-π-A⁺-π-D structure, where A stands for the quinolizinium cation acceptor group and D stands either for dimethyl aniline or methoxyphenyl donor groups. Here we want to report on the evaluation of the application of the best performing system as a label for in vivo nonlinear optical microscopy. The two synthesized cationic V-shaped molecules (E,E)-2,8-bis(4-methoxyphenylvinyl)quinolizinium and (E,E)-2,8-bis(4,N,N-dimethylaminophenylvinyl)quinolizinium hexafluorophosphate, hereby denoted as V-MOP2 and V-dma2, were obtained in very high yield from 2,8-dimethylquinolizinium bromide by a double Knoevenagel condensation, as illustrated in Scheme 1 (details of the synthesis are provided in S2–S4, ESI†).

The photophysics of V-MOP2 and V-dma2 have been characterized in methanol. A summary of the results is given in Table 1. The linear absorption and emission spectra are shown in Fig. 1. V-MOP2 and V-DMA2 are green and red emitters, respectively, with two-photon absorption cross-section maxima at 760 and 880 nm, well within the bandwidth of the Ti:Sapphire laser (the most common pulsed laser used in multiphoton fluorescence microscopy). A blueshift of

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Scheme 1 Synthesis of (E,E)-2,8-bis(4-methoxyphenylvinyl)quinolizinium (V-MOP2) and (E,E)-2,8-bis(4,N,N-dimethylaminophenylvinyl)quinolizinium hexafluorophosphate (V-DMA2).
Table 1 Photophysical properties of V-MOP2 and V-DMA2 in methanol solution

<table>
<thead>
<tr>
<th>Compound</th>
<th>V-MOP2</th>
<th>V-DMA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{abs} ) nm OP</td>
<td>432</td>
<td>518</td>
</tr>
<tr>
<td>( \lambda_{abs} ) nm TP</td>
<td>760</td>
<td>880</td>
</tr>
<tr>
<td>( \lambda_{em} ) nm OP</td>
<td>546</td>
<td>650</td>
</tr>
<tr>
<td>( \sigma_2 ) GM (^a)</td>
<td>1144</td>
<td>341</td>
</tr>
<tr>
<td>( \phi )</td>
<td>0.22</td>
<td>0.003</td>
</tr>
</tbody>
</table>

\(^a\) OP and TP stand for one-photon and two-photon maxima, respectively. \(^b\) Determined by two-photon stimulated emission using an aqueous solution of fluorescein at pH 11 as a standard. \(^c\) Details of the experimental setup are provided in S3 (ESI).
The authors acknowledge financial support from the Spanish Ministerio de Ciencia e Innovación (project CTQ2008-04313/BQU), the Portuguese Fundação para a Ciência e a Tecnologia (project PTDC/CTM-POL/114367/2009) and a grant from the Universidad de Alcalá (TC).

Notes and references

19 A change in the counterion of these quinolizinium salts can improve water solubility as observed for iodide or bromide salts.