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Syntheses and properties of second-generation V-shaped xanthene dyes with piperidino groups

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ABSTRACT

New V-shaped xanthene dyes **3**–**6** containing piperidino groups at the resonance sites on their skeletons were constructed as second-generation V-shaped dyes and their functions were investigated. The non-symmetric compound **5** had higher solubility and gave a higher fluorescence quantum yield and longer emission wavelength compared with those of previously reported V-shaped xanthene dyes with hydroxyl groups **1** and **2**. Furthermore, compound **5** stained living cells, enabling bright fluorescence imaging.

1. Introduction

In the field of molecular biology, fluorescent dyes are essential tools for visualizing target living organisms in real time.[1] Fluorescein was first synthesized by Bayer in 1871,[2] and since then various fluorescent dyes based on the xanthene framework have been developed.[3] Nagano and Urano developed TokyoGreen and showed that the upper aryl moiety at the 9-position of the xanthene skeleton is orthogonal to the bottom xanthene plane, and the upper aryl group does not affect the fluorescence emission wavelength. The upper aryl moiety is therefore mainly used as a scaffold for photoinduced electron transfer, donor-excited photoinduced electron transfer, and/or conjugation sites for target organs.[4]

We developed naphthofluorescein derivatives by adding benzene rings to the parent fluorescein skeleton, to expand the π−system and give a longer emission wavelength.[5] Large red shifts of both the absorption and emission wavelengths were achieved using this method, but the solubilities and fluorescence quantum yields ($\Phi < 1\%$) decreased. Instead of adding benzene rings, we therefore constructed V-shaped xanthene dyes **1** and **2**, in which the upper aryl group is connected to the bottom xanthene part by an ether linkage and the π−system is extended across the molecule (Fig. 1).[6,7]

The V-shaped compounds **1** and **2** showed four colors, depending on whether an acid or a base was added, because of the complicated π−resonance system involving two hydroxyl groups and one carbonyl group. In particular, the emission of **2** in DMSO was observed at 595 nm, and reached the red emission region. These results indicate that V-shaped compounds have potential applications as new longer-emission-wavelength fluorescent dyes. However, some of the properties of **2** need to be improved, i.e., its poor solubility and moderate fluorescence quantum yield ($\Phi = 10\%$).

In this study, we synthesized new rhodol[3d,e] and rhodamine-type[3] V-shaped fluorescent dyes containing one to three piperidino groups (Fig. 2), and investigated their use in imaging of living cells. The improvements needed in the properties of these compounds are (1) longer emission wavelengths, (2) higher quantum yields, and (3) higher solubilities.

2. Syntheses of V-shaped xanthene dyes 3–6

The synthetic routes used to obtain V-shaped xanthene dyes **3**–**6** are shown in Scheme 1. First, the two hydroxyl groups in **1**

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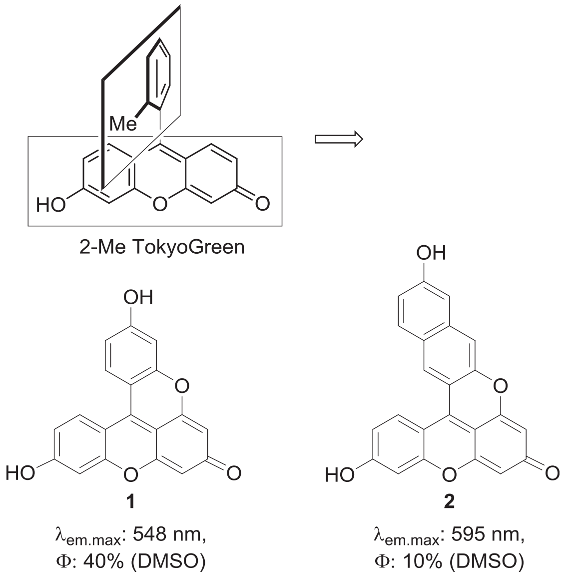


Fig. 1. V-shaped xanthene dyes **1** and **2**.

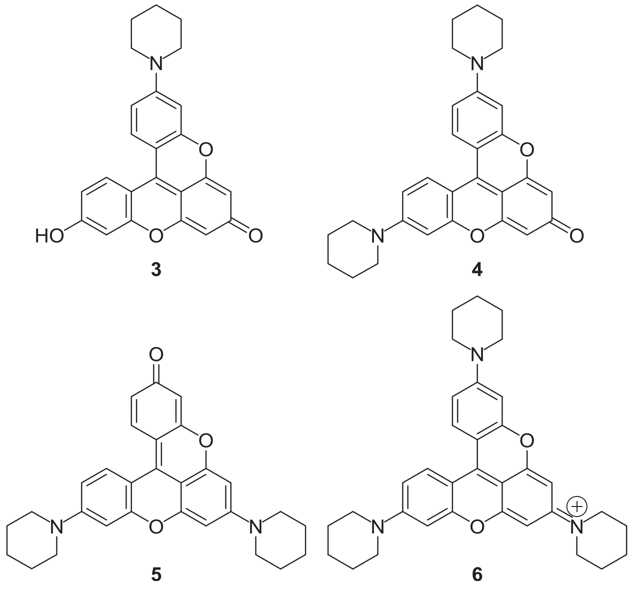
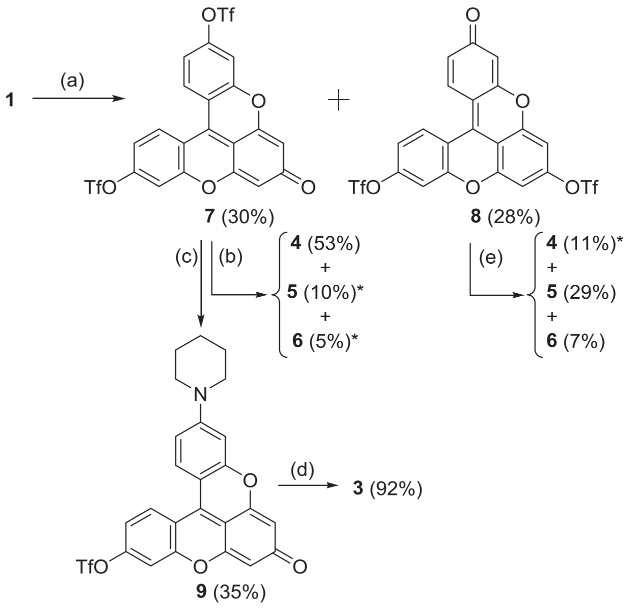


Fig. 2. Structures of piperidine-containing V-shaped xanthene dyes **3**–**6**.



Scheme 1. Synthesis of V-shaped dyes **3**, **4**, **5**, and **6**. Conditions: (a) $PhN(Tf)_2$ (2.4 equiv), DIPEA (6.0 equiv); (b) piperidine (16 equiv); (c) piperidine (1 equiv); (d) i) NaOH aq (3 equiv) and ii) Amberlyst 15 (3 equiv); (e) piperidine (10 equiv). * Yield was calculated from crude reaction mixture using ^1H NMR spectroscopy (NMR yield).

were converted to the corresponding triflates using $PhN(Tf)_2$ and Hünig's base, affording regioisomeric compounds **7** and **8** in 30% and 28% yields, respectively.[8] Compound **4** was synthesized in 53% yield from **7** by aromatic nucleophilic substitution with excess piperidine.[3a] Generation of regioisomer **5** and the overreacted product **6** was also observed, and their NMR yields were estimated to be 10% and 5%, respectively. When the reaction was performed using **7** and 1 equiv of piperidine, to prevent the formation of the overreacted products of **4** and **5**, which are difficult to separate, the monosubstituted compound **9** was obtained in 35% yield, along with 52% recovery of **7**. The triflate group in **9** was deprotected under basic conditions and then treated with an acidic ion-exchange resin to obtain the desired compound **3** in 92% yield. Treatment of **8** with a large excess of piperidine gave the disubstituted compound **5** (29% yield), trisubstituted compound **6** (7% yield), and the migration compound **4** (not isolated, 11% NMR yield) after repeated column chromatography.

3. Optical properties of V-shaped xanthene dyes 3–6

Similarly to previously reported V-shaped xanthene dyes **1** and **2**, compounds **3**–**6**, which contain phenolic hydroxyl and piperidino groups, can change form in response to an acid or base. Fig. 3 shows the behavior of **5** on addition of an acid (for the optical properties of the other V-shaped dyes, see the Supplementary Material). When a DMSO solution of **5** (1×10^{-5} M) was titrated with acetic acid, the intensities of the absorptions at 542 and 506 nm gradually decreased and that of the absorption at 558 nm increased, with isosbestic points at 318 and 549 nm (Fig. 3). The addition of 30,000 equiv of acetic acid was needed to reach a plateau; the UV spectrum was almost unaffected by subsequent addition of a small amount of hydrochloric acid (10 equiv). In contrast, when tetrabutylammonium hydroxide solution (10 equiv) was added to a DMSO solution of **5**, the UV spectrum of **5** did not change. These results suggest that **5** was present in its neutral form in DMSO solution, but existed in its cationic form in the presence of a large excess of acetic acid. The UV and fluorescence spectra of the neutral and cationic forms of **5** were recorded under these conditions, and the fluorescence quantum yields were determined (Fig. 4). Compound **4** also existed in two forms (neutral and cationic); compound **3**, which contained both phenolic hydroxyl and piperidino groups, had three forms (cationic, neutral, and anionic). Compound **6** gave broad absorption and emission spectra (see the Supplementary Material).

Fig. 5 shows the normalized fluorescence spectra of the cationic forms of **1**, **3**, **4**, **5**, and **6** in DMSO; characteristic parameters, i.e., λ_{max} for absorbance, excitation, and emission, quantum yields (Φ), and Stokes shifts of V-shaped dyes **1**–**6** (cationic forms) are summarized in Table 1, with images obtained under white and black (365 nm) lights.

The emission wavelengths of all the new dyes **3**–**6** with piperidino groups were longer than that of **1**. Compounds **3**–**6** gave