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Development of xanthene dyes containing arylacetylenes: The role of acetylene linker and substituents on the aryl group

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ABSTRACT

Fluorescent dyes possessing a variety of arylacetylenes at the 9-position of a xanthene skeleton were synthesized and their optical properties were investigated. The π system effectively expanded over the xanthene skeleton and the aryl group through the triple bond. Starting from the emission wavelength (λ_{em}) of 9-methyl xanthene **20** in basic DMSO solution at 536 nm, the emission wavelengths gradually shifted to the red region for methylacetylene **17** ($\lambda_{em} = 600$ nm), phenylacetylene **5** ($\lambda_{em} = 636$ nm), and *p*-CF_3-phenylacetylene **11** ($\lambda_{em} = 660$ nm). On the basis of these data, we estimated the substituent effects for the red shift on the emission wavelength and rationally explained the results by DFT calculations. Furthermore, potential applicability of these fluorescent dyes to cell staining was exemplified.

1. Introduction

Xanthene-based fluorescent dyes represented by fluorescein (**1**)[1] have been widely used in the fields of molecular biology and medicinal chemistry.[2] Fluorescein and/or xanthene-based fluorescent dyes usually show emission at approximately 510 nm owing to the use of the xanthene skeleton as a common fluorophore.[3] To avoid overlap with the auto fluorescence of living organisms such as tryptophan and heme, many attempts have been reported to shift the emission wavelength of fluorescent dyes into the red or near infrared regions. In particular, the conversion of the oxygen at the 10-position of xanthenes to silicon[4] or phosphorus[5] has been shown to be effective for these purposes and widely investigated.

We have also been studying fluorescent dyes that show emission in the red/near infrared region based on xanthene frameworks by alternative approaches (Fig. 1). First, we synthesized dinaphthofluorescein **2**, which features an expanded π-conjugate system, by the simple addition of benzene rings to both sides of fluorescein.[6] Compound **2** showed emission at 790 nm as expected; however, this molecule featured quite a low solubility in organic solvents and water with a low fluorescence quantum yield ($\Phi \leq 0.1\%$). On the basis of these results, we have tried to develop new fluorescent molecules that have a molecular weight comparable to that of fluorescein, but show absorption and emission at longer wavelengths than those of fluorescein. We recently developed V-shaped xanthene dyes **3**[7] and **4**,[8] in which a xanthene skeleton and an aryl group are connected with an ether linkage, and the aryl group is incorporated into the π-system of the xanthene skeleton. The V-shaped **3** showed emission at wavelengths of 550 nm, reflecting a 40 nm red shift compared with that of fluorescein.

In this manuscript, we will demonstrate an alternative approach, which involves connecting the aryl group to the xanthene skeleton through a triple bond, to achieve large red shifts in both the absorption and emission wavelengths. Fluorescein and its derivatives generally consist of a xanthene skeleton and an aryl group at 9-position, which although orthogonal to xanthene system does subtly interact. However, the acetylene linkages between the two moieties are in the same plane and expansion of the π system might be expected. Although, four similar fluorescent dyes connecting aryl groups and (pseudo)xanthene skeletons with acetylene moieties have been reported in patents[9] and papers,[10] only phenyl and mesityl groups were investigated as the aryl groups (Fig. 2). We believe that more extensive research on different substituents effects on the aryl groups and the role of acetylene linker could be useful, together with computational studies. In this study, we synthesized a variety of acetylene bridged

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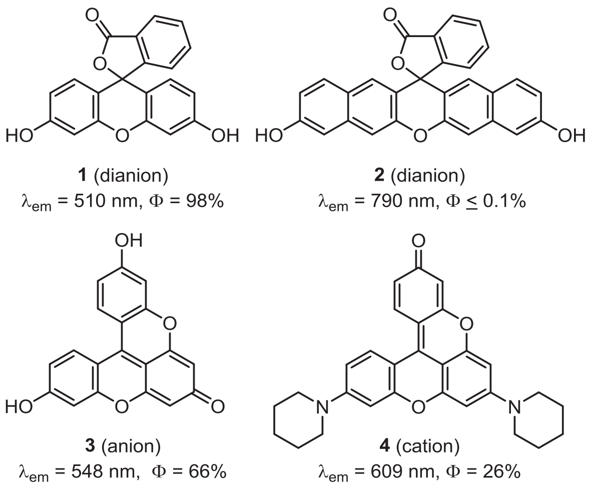
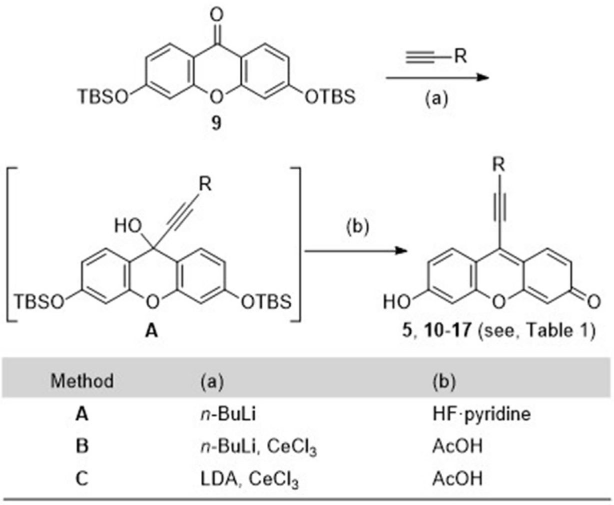

1 (dianion)
λ_{em} = 510 nm, Φ = 98%
2 (dianion)
λ_{em} = 790 nm, $\Phi \leq 0.1\%$
3 (anion)
λ_{em} = 548 nm, Φ = 66%
4 (cation)
λ_{em} = 609 nm, Φ = 26%

Fig. 1. Structures of fluorescent dyes **1**–**4**.



Method	(a)	(b)
A	*n*-BuLi	HF·pyridine
B	*n*-BuLi, $CeCl_3$	AcOH
C	LDA, $CeCl_3$	AcOH

Scheme 1. Synthesis of acetylene linked dyes **5** and **10**–**17**.

xanthene dyes and revealed their photophysical properties, including substituent effects on the aryl group and solvatochromism, for applications to imaging of living cells.

1.1. Syntheses of acetylene bridged xanthene dyes 5 and 10–17

First, the acetylene bridged xanthene dyes **5** and **10**–**17** were synthesized according to a reported procedure (Scheme 1, method A).[9] Thus, the arylacetylenes were treated with *n*-BuLi to prepare corresponding lithium acetylides. These were then reacted with TBS protected xanthone **9**[11] to give intermediate **A**, followed by removal of the two TBS groups under HF-pyridine conditions to afford the desired acetylene bridged xanthene dyes **5**, **10**, and **11**[12] in yields of 67%, 83%, and 21%, respectively. However, in the case of a combination of lithium *p*-F-phenylacetylide and **9**, a trace amount of desired **12** was obtained, and deprotected xanthone **18** and TBS acetylene **19** were isolated in yields of 78% and 23%, respectively. Thus, the lithium acetylide predominantly attacked the silicon atoms of the protecting TBS group. These data indicate that the nucleophilicity of lithium acetylide possessing an electron withdrawing substituent on the aryl group was reduced, and its soft character was increased. To improve the selectivity and reactivity of lithium acetylide, anhydrous cerium chloride was added to the solution to generate the corresponding cerium acetylide in situ, according to Imamoto's procedure.[13] The cerium acetylide reacted smoothly with the carbonyl group on compound **9**, and the following deprotection of the two TBS groups of the phenolic hydroxy groups was performed under acetic acid conditions to afford the desired acetylene bridged xanthenes **11**–**17** in moderate to high yields (Method B). Owing to the low yield of the 2-pyridiyl acetylene compound **15** (35%) obtained with the use of method B, a combination of LDA and $CeCl_3$ was used to obtain **15** in 65% yield (Method C). The yields of the products and the applied synthetic methods are summarized in Table 1.

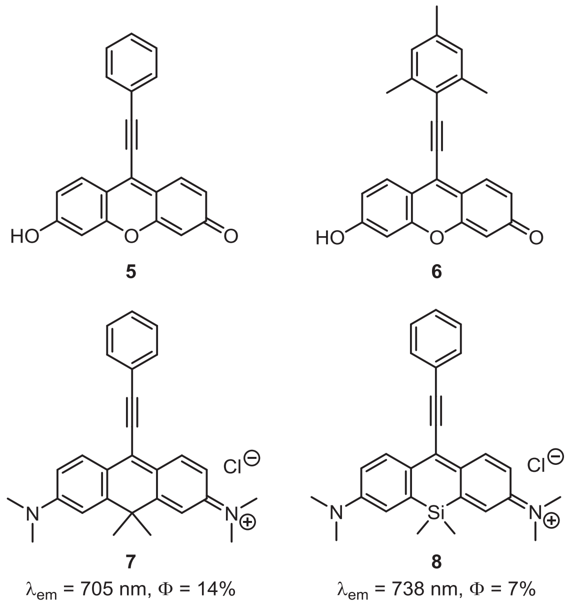
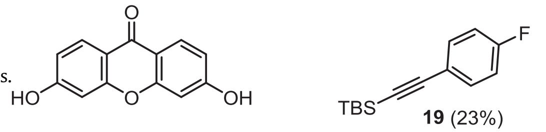

5
6
7
λ_{em} = 705 nm, Φ = 14%
8
λ_{em} = 738 nm, Φ = 7%

Fig. 2. Four reported (pseudo)xanthene dyes with arylacetylene moieties.

Table 1
Yields and applied methods.

Entry	Product	R	Method	Yield (%)
1	**5**	C_6H_5	A	67
2	**10**	*p*-OMe-C_6H_4	A	83
3	**11**	*p*-CF_3–C_6H_4	A	21
4			B	53
5	**12**	*p*-F–C_6H_4	A	trace[a]
6			B	60
7	**13**	*m*-F–C_6H_4	B	69
8	**14**	*o*-F–C_6H_4	B	68
9	**15**	2-pyridyl	B	35
10			C	65
11	**16**	TMS	B	58
12	**17**	Me	B	65

[a] Compounds **18** and **19** were isolated as by-products.


18 (78%)
19 (23%)