HETEROCYCLES, Vol. 103, No. 1, 2021, pp. 365 - 378. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 4th June, 2020, Accepted, 31st July, 2020, Published online, 9th September, 2020
DOI: 10.3987/COM-20-S(K)19

AN ALTERNATIVE APPROACH TO THE DEVELOPMENT OF NEAR-INFRARED FLUORESCENT DYES BASED ON FLUORESCEIN

Akane Katori,[a] Akari Yamagami,[a] Sae Wakabayashi,[a] Kouji Kuramochi,[b] Ayumi Imayoshi,[a] and Kazunori Tsubaki [a]*

a) Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606-8522, Japan. b) Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan. E-mail: tsubaki@kpu.ac.jp

This paper is dedicated to Professor Yasuyuki Kita in celebration of his 77th birthday.

Abstract – In xanthene dyes the upper aryl moiety is often a benzene ring, but in this work an L-shaped dibenzoxanthene dye (**4**) was synthesized with a five-membered (benz)imidazole ring as the upper aryl moiety. Although **4** was designed with the π-face of the dibenzoxanthene and the π-plane of the aryl moiety as coplanar, the reactivity of the compound at the triaryl carbon was quite high and easily attacked by solvent, such as methanol. The consequent discoloration is caused not only by the external exposure of the triaryl carbon, but also by the greatly reduced LUMO level and increased reactivity to nucleophilic attack when the π-system of the xanthene ring is expanded. The introduction of the 2,6-dimethylphenyl group was found to be effective to avoid nucleophilic attack by solvent. A compound, labeled **9c**, with a 2,6-dimethylphenyl group as the aryl moiety, had sufficient stability to solvent, an emission maximum at 828 nm in DMF, and a fluorescence quantum yield of 8%.

INTRODUCTION

Fluorescein was synthesized by Bayer about 150 years ago, and the system still receives much attention from analytical chemists and chemical biologists.[1] Recently, while maintaining the excellent features of fluorescein, such as its high quantum yield and wide solvent applicability, many synthetic efforts toward shifting the excitation and emission wavelengths to the near infrared region have been attempted to avoid the overlap of autofluorescence from the living organism.[2] For a simple route to achieve this purpose, π-system-expanded fluoresceins were developed, which have condensed additional benzene rings to one or both sides of the fluorescein. For example, Strongin *et al*. synthesized a U-shaped dinaphthofluorescein,[3] and seminaphthofluorescein (SNAFL) derivatives.[4]

We developed syntheses of nine π-system-expanded (di)naphthofluoresceins (including the above known compounds) and evaluated their optical properties.[5,6] As a result, we revealed a tendency that (i) expansion of two benzene rings to the side of the basic xanthene skeleton provides a longer (by about 200 nm) emission wavelength shift and (ii) expansion of the benzene ring downward (in the direction relative to the xanthene oxygen) leads to a relatively high quantum yield (Figure 1).

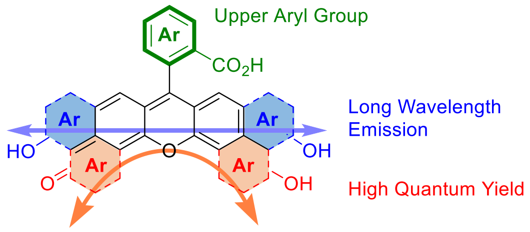


Figure 1. Structure-function relationship of π-expanded xanthene dyes

When two benzene rings are added to the xanthene ring, the produced L-shaped dyes, which have one benzene ring added to the side of the xanthene and another to its downward direction, are expected to give preferred performance. Based on these findings, we designed new molecules in which the emission toward the near infrared region was determined by the relationship between the xanthene part and aryl moiety. Because the aryl fragment of xanthene dye is usually perpendicular to the xanthene π-system and is used as a scaffold in FRET systems, the π-interaction between the upper and lower π-planes is negligible.[7] If the xanthene ring and the aryl moiety can be arranged in a parallel fashion, *i.e*., if the dihedral angle of two rings can be arranged to be more acute, it is possible to construct a large π-system spread over the xanthene ring and the aryl moiety. It is also expected that the absorption and emission wavelengths of these compounds will be largely shifted to a longer wavelength. With this concept, we have developed (i) V-shaped dyes that connect the two rings by ethereal covalent bonds,[8,9] and (ii) dyes that connect the two rings through triple bonds.[10] As expected, the absorption and emission wavelengths of these dyes successfully shifted to a longer wavelength for each design. For the third design, and along similar lines to this concept, we report in this paper on the development of dyes in which the xanthene ring is linked by a five-membered aromatic ring (imidazole) to reduce steric repulsion between the aryl moiety and xanthene ring.

RESULTS AND DISCUSSION

First, we calculated the most stable conformations of the anionic form of compound **A** (xanthene possessing a phenyl group) and the anionic compound **B** (xanthene having an imidazole group) as simple model molecules. The UV-vis spectra of anionic **B** was calculated as the dihedral angle was varied from 0° to 90° in steps of 10° (Figure 2).[11] The calculations indicated that, in the ground state, the most stable conformation of **A**$^-$ is when the dihedral angle between the xanthene ring and phenyl group is 71.9°, while the equivalent dihedral for the most stable conformation of **B**$^-$ is about 52.1°. This is because of a reduction in the steric repulsion of the hydrogen atoms on the xanthene ring and hydrogen atoms on the aryl ring (shown in red in Figure 2a). In addition, as the dihedral angle of **B**$^-$ becomes 0°, the absorption wavelength is expected to shift to a longer wavelength (Figure 2b).

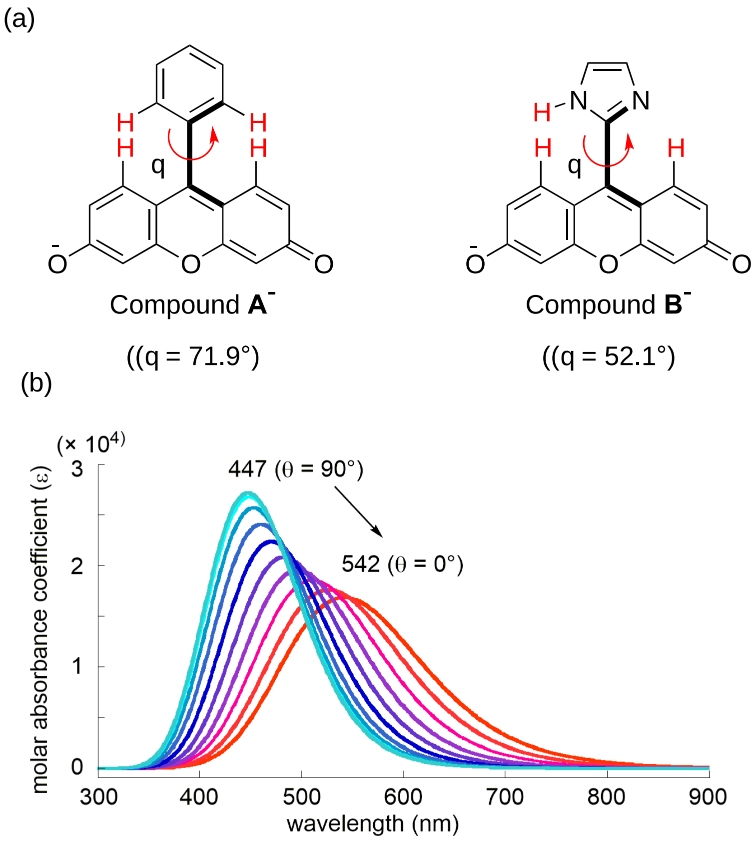
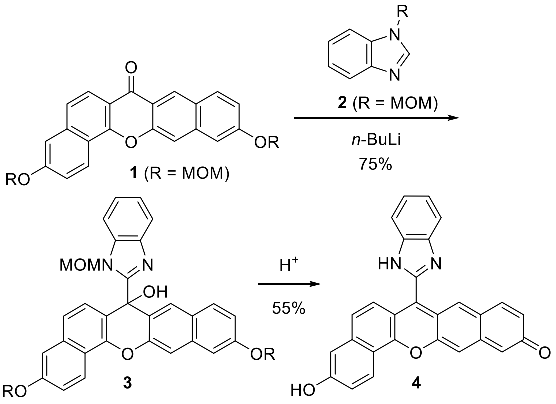


Figure 2. (a) Structures of compounds **A**$^-$ and **B**$^-$ and (b) calculated UV-vis spectra of **B**$^-$ varying the dihedral angle (θ) between xanthene and imidazole. The spectra were obtained by TD-DFT calculations at the B3LYP/6-31+G(d,p) level and by fitting the predicted oscillators with 0.333 eV half-width at half height.

Using the same strategy, compound **4** was synthesized, possessing benzimidazole, and gave the expected enhanced π-expansion (Scheme 1). The MOM-protected benzimidazole **2** was treated with *n*-BuLi followed by reaction with L-shaped dibenzoxanthone **1**[5] to give compound **3** in 75% yield. Three MOM groups of **3** were removed by acidic treatment to afford desired compound **4** in 55% yield.



Scheme 1. Synthesis of compound **4**

UV-vis spectra of compound **4** in acidic, neutral, and basic media are shown in Figure 3. In acidic medium, compound **4** gave a bright purple color and showed an absorption maximum at 554 nm. In contrast, in neutral and basic media, compound **4** showed no apparent absorption.

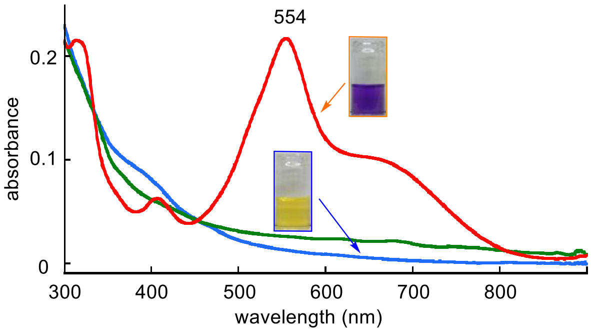
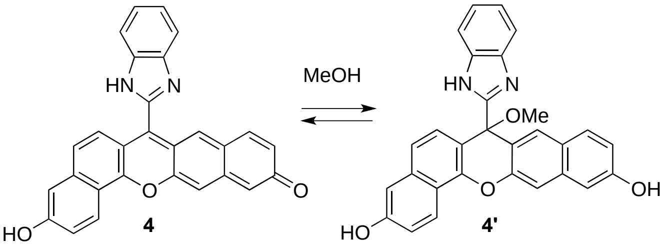


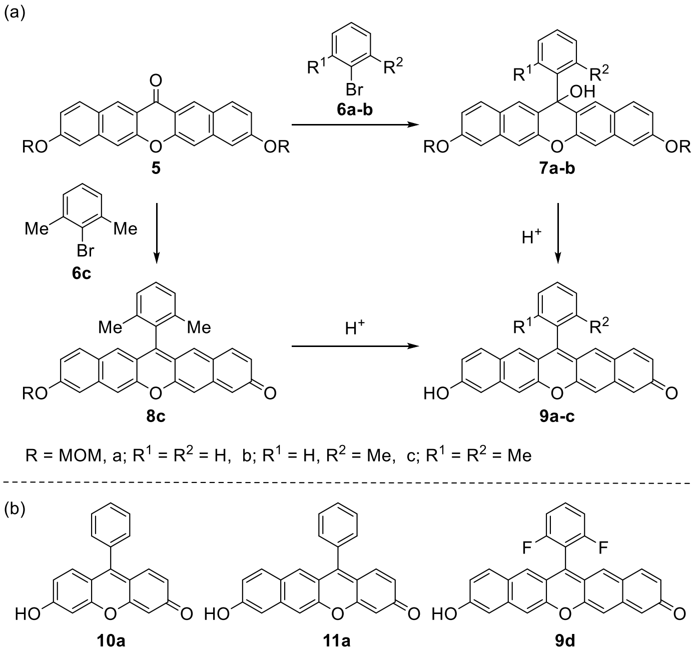
Figure 3. UV-vis spectra of compound **4** in aqueous solutions. Conditions: pH 0 ($MeSO_3H$, red), pH 7 (citrate-phosphate buffer, green), pH 11 (Glycine-NaOH buffer, blue), concentration = 2×10^{-5} M, temp = 25 °C, light pass length = 10 mm.



Scheme 2. Reversible reaction of compound **4** and methanol

The reason for the disappointing results for compound **4** became clear through NMR measurements in methanol-*d*$_4$. When compound **4** was added to methanol-*d*$_4$, a bright purple color was immediately bleached. Further, as the solvent was removed under reduced pressure, the purple color went reversibly back. These results clearly indicated that a methanol molecule easily attacked the triaryl carbon of the dye to cut its conjugation system. Thus, as coplanarity between the upper and lower fragments is increased by introducing benzimidazole, the highly reactive triaryl carbon is exposed on the outside and the carbon is vulnerable to nucleophilic attack by solvent molecules (Scheme 2). Therefore, our third strategy does not work and steric protection around the triaryl carbon is necessary.

As another factor, we speculated that this easy decoloration might be related to the number of aromatic rings on the xanthene ring. To verify the relationship between the number of aromatic rings of the xanthene fragment and the bulkiness around the triaryl carbon, we synthesized a series of dibenzoxanthenes, **9a–c**, possessing phenyl, 2-methylphenyl, and 2,6-dimethylphenyl groups at the triaryl carbon, respectively (Scheme 3).[12] We also prepared tricyclic xanthene **10a**[13] and tetracyclic (mono)benzoxanthene **11a**[4] as reference compounds (Scheme 3b). Compounds **9a–c** were prepared through a similar synthetic approach as for compound **4**. Thus, the MOM-protected dibenzoxanthone **5**[5] was treated with the corresponding aryllithium-derived from arylbromide **6a–b** and *n*-BuLi to afford the trityl alcohols **7a** in 91% yield and **7b** in 65% yield, respectively. After acidic deprotection of the MOM groups and dehydration for compounds **7a** and **7b**, desired compounds **9a–b** were obtained in 71%, and 69% yields, respectively.



Scheme 3. (a) Syntheses of compounds **9a–c**. (b) Reference compounds **10a**, **11a**, and **9d**.

For the combination of compound **5** and **6c**, mono-deprotected **8c** was obtained in 50% yield. After deprotection of the MOM group for **8c**, desired compounds **9c** was afforded in 55% yields.

First, the stabilities of the synthesized dyes **9a–c**, **10a**, and **11a** were evaluated. The time-course measurements of absorbance at λ_{max} of the dye was monitored in methanol at 25 °C and the half-life of the dye was determined through its decay curve (Figure 4). Compound **9c**, possessing a 2,6-dimethylphenyl group, showed no color change in absorbance during 11 h. In contrast, compound **9b**, having a 2-methylphenyl group, indicated a moderate decay curve and $t_{1/2}$ was estimated for 2.2 h. Compound **9a**, bearing a phenyl group, was immediately breached by methanol, even in dichloromethane solution containing 0.5% methanol as stabilizer, and $t_{1/2}$ was estimated to only be 356 s. In addition, compounds **10a** and **11a**, which possess no methyl group on the aryl moieties, showed no deterioration of color.

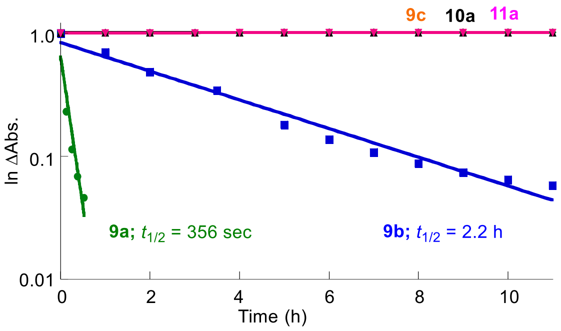


Figure 4. Determination of half-life of **9a–c**, **10a**, and **11a** (1×10^{-4} M) in MeOH. For compound **9a**, the decay curve was monitored in CH_2Cl_2 containing 0.5% MeOH as a stabilizer.

A comparison of compounds **9a** through **11a** shows that the number of aromatic rings constituting the xanthene fragment greatly affects the reactivity at the triaryl carbon. Thus, as the π-system of the xanthene spreads, the reactivity of the triaryl carbon is increased and is easily attacked by solvent. Therefore, in the case of the dibenzoxanthene system, to kinetically prevent the nucleophilic addition of solvent, at minimum, a perpendicular 2,6-dimethylphenyl group as the upper fragment is essential.

The difference in reactivity with the number of constituent aromatic rings of the xanthene fragment was evaluated by computational calculations.[11] Figure 5 shows the HOMO−LUMO levels and their gaps for the anionic forms of compounds **9a** through to **11a**. As the number of aromatic rings increases, the HOMO−LUMO gap becomes smaller. This is mainly caused by the decrease in the LUMO level. Therefore, it is for this reason that compound **9a** is easily attacked by the solvent molecule.

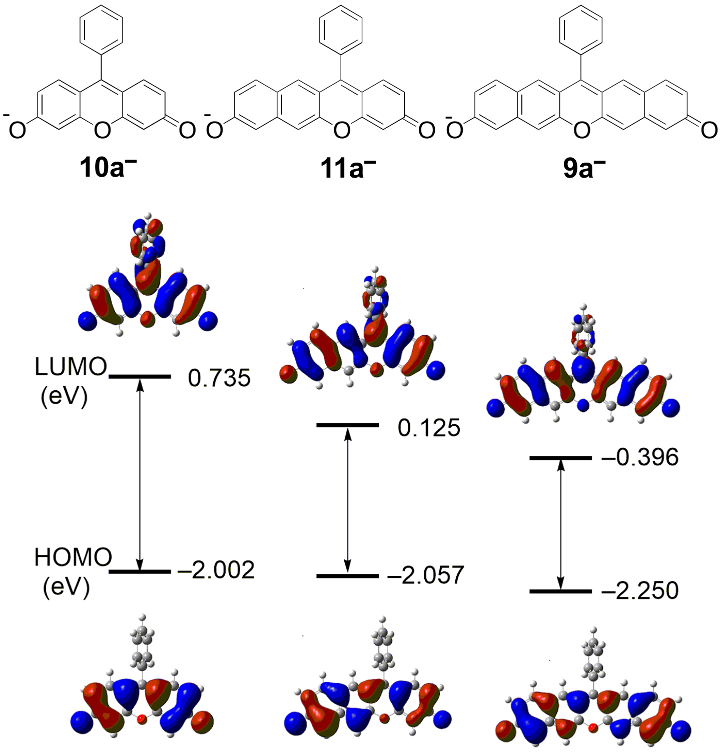


Figure 5. HOMOs, LUMOs, and energy gaps for anionic **10a**, **11a**, and **9a**, calculated using DFT at the B3LYP/6-31+G(d,p) level.

Aratani and Yamada *et al*. also synthesized compound **9a** and reported that the triaryl carbon of **9a** was readily attacked by solvents, such as water and methanol.[14] Therefore, they synthesized compound **9d**, which possesses two fluorines at the ortho position of the upper phenyl group, to prevent the reaction with solvent molecule and evaluated its optical properties. Compound **9d** showed sufficient stability against solvent, but it is gradually decomposed by light and air. In our experiments, compound **9a** was somewhat reactive to nucleophilic attack by solvents, while compound **9c**, protected at the triaryl position with two methyl groups, showed sufficient stability against solvent. Thus, our results are consistent with their data.

The optical properties of compound **9c** were measured because it was sufficiently resistant to nucleophilic solvent (Figure 6 and Table 1). The UV-vis and fluorescence spectra of compound **9c** were taken in various solvents, including a small amount of aqueous NaOH solution. Even in a basic buffer (pH 11) and basic methanol, compound **9c** showed clear absorptions at 723 and 750 nm, respectively. However, the fluorescence spectra of **9c** were vague in shape and weak in signal intensity, while peak tops were barely observed at 778 nm (in buffer) and 788 nm (in methanol), and the fluorescence quantum yields were estimated to be around 0.24% and 1.7%, respectively. In contrast, in basic DMSO solution, compound **9c** clearly indicated an emission maximum at 830 nm with small Stokes shifts (33 nm) and quantum yields of about 5.7%. Furthermore, compound **9c** also exerted its potency in DMF solution (λ_{abs}, 795 nm; λ_{em}, 828; Φ = 8%). Compound **9c** exhibited peak tops in both its absorption and fluorescence spectra at longer wavelengths in aprotic DMSO and DMF, compared with in protic buffer and methanol. These data are ascribed by the anionic **9c** being strongly hydrogen-bonded and largely stabilized in the ground state with protic solvent, and the interaction would be maintained (although weakened) in the exited state. As a result, the nonradiative path takes precedence. However, in aprotic solvents, the interaction with the solvents would be minimal in both the ground and excited states, and the intrinsic performance of the compound **9c** would be inhibited.

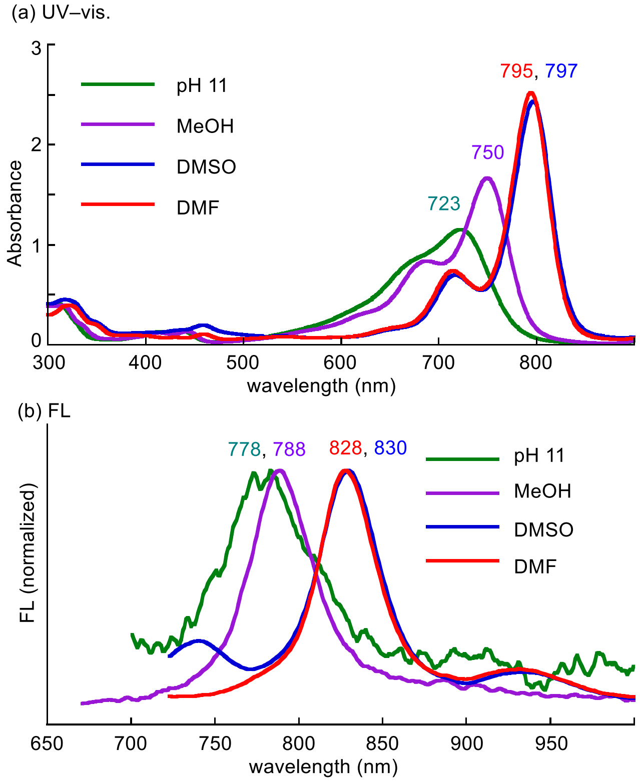


Figure 6. (a) UV-vis spectra and (b) fluorescence spectra (normalized) of compound **9c** in various solvents under basic conditions. Concentration = 2×10^{-5} M; temp = 25 °C; light pass length = 10 mm.

Table 1. Photophysical properties of compound **9c**

solvent	$\lambda_{\mathrm{abs.Max}}$	$\lambda_{\mathrm{em.Max}}$	Stokes Shift (nm)	Φ (%)[a]
Buffer (pH 11)	723	778	55	0.24
MeOH	750	788	38	1.7
DMSO	797	830	33	5.7
DMF	795	828	33	8.0

Each spectrum was measured in Glycine-NaOH buffer (pH 11), DMSO, DMF, or MeOH, containing 500 equiv. of NaOH. Temp. = 25 °C, light pass length = 10 mm. [a]Based on indocyanine green as a reference standard (Φ = 12%).[15]

In conclusion, we synthesized a fluorescent dye in which the upper aryl moiety of fluorescein was converted from a benzene ring to a benzimidazole ring. This transformation increases the coplanarity of the xanthene ring and the upper aryl moiety and broadens the π-system of the molecule. As a result, absorption and emission at the longer wavelength region is expected. However, this approach did not work well because the triaryl carbon was exposed to the outside as the two aromatic rings became coplanar, and was easily attacked by solvent, such as water and methanol. A series of xanthene dyes were then synthesized in which the upper aryl moiety was fixed to the benzene ring, and additional benzene rings were added to the lower xanthene segment. It was found that in the cases of tricyclic xanthene and tetracyclic benzoxanthene skeletons, these dyes had sufficient stability against surrounding solvents, even for the case of a nonsubstituted phenyl group as the upper aryl moiety. However, for the combination of pentacyclic dibenzoxanthene skeleton and phenyl group, the triaryl carbon was easily attacked by solvent because of the reduced LUMO level of the molecule. To block nucleophilic attack by solvent, the introduction of two methyl groups into the upper benzene ring was essential. It was also found that dibenzoxanthene dye had emission in the near infrared region, and the quantum yields were largely dependent on the solvent used. These findings will greatly contribute to the further improvement of fluorescein-type dyes.

EXPERIMENTAL

Compound **3**. A solution of *n*-BuLi (1.60 M *n*-hexane solution; 0.186 mL, 0.298 mmol) was added dropwise over 3 min to a solution of **2** (46.2 mg, 0.288 mmol,) in dry THF (3 mL) under nitrogen at –78 °C. After stirring for 2.5 h, a solution of **1** (80.0 mg, 0.192 mmol) in dry THF (1.2 mL) was added dropwise to the reaction mixture, and the resulting solution was stirred at –78 °C for 80 min. The solution was poured into EtOAc and water. The organic layer was separated and washed sequentially with water (three times) and brine, before being dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The resulting residue was purified by column chromatography (SiO_2; *n*-hexane/EtOAc = 4:1) to afford compound **3** (82.8 mg, 75% yield) as pale yellow oil.; IR ($CHCl_3$) 3398, 2956, 2902, 2827, 1637, 1610, 1585, 1506, 1475, 1414, 1396, 1354, 1255, 1147, 1076, 1003, 924, 868, 752 cm^{-1}; ^1H-NMR; (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 8.8 Hz, 1H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.80 (s, 1H), 7.76 (s, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 7.42-7.36 (m, 6H), 7.29 (td, *J* = 7.6 Hz, 0.8 Hz, 1H), 7.06 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.89 (s, 1H), 5.33-5.31 (m, 4H), 4.71 (d, *J* = 2.4 Hz, 2H), 3.54 (s, 3H), 3.53 (s, 3H), 2.21 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.9, 156.5, 156.2, 148.2, 145.7, 140.1, 136.8, 135.8, 135.4, 129.8, 129.2, 126.6, 125.7, 124.0, 123.7, 123.1, 122.6, 122.3, 119.9, 119.4, 119.0, 118.4, 114.4, 111.5, 111.3, 110.1, 108.2, 94.5, 94.4, 75.1, 67.0, 56.2, 56.2, 55.1; HRMS (ESI) calcd for $C_{34}H_{30}N_2O_7Na$ $(M+Na)^+$ 601.1935, Found 601.1945.

Compound **4**. A solution of 4 M hydrogen chloride in 1,4-dioxane (0.40 mL) was added dropwise to a solution of **3** (31.0 mg, 0.053 mmol) in 1,4-dioxane (0.40 mL) and stirred for 20 h at room temperature. The solution was poured into EtOAc and water. The organic layer was separated and washed successively with water (three times) and brine, before being dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The resulting residue was washed with hexane and then purified by column chromatography (SiO_2; *n*-hexane/EtOAc = 1:1) to afford compound **4** (12.7 mg, 55% yield) as a purple powder.; mp >300 °C; IR (KBr) 3728, 3396, 1635, 1601, 1520, 1475, 1452, 1394, 1319, 1284, 1238, 1213, 1176, 1147 cm^{-1}; ^{1}H-NMR; (400 MHz, CD_3OD, **4** MeOH adduct) δ 8.55 (d, *J* = 9.2 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.86 (s, 2H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.74 (td, *J* = 8.4 Hz, 0.8 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.64 (td, *J* = 8.0 Hz, 0.8 Hz, 1H), 7.49 (d, *J* = 9.2 Hz, 1H), 7.32 (dd, *J* = 8.8 Hz, 3.0 Hz, 1H), 7.21 (d, *J* = 3.0 Hz, 1H), 7.18 (d, *J* = 3.0 Hz, 1H), 7.15 (d, *J* = 8.8 Hz, 1H), 7.04 (dd, *J* = 8.8 Hz, 3.0 Hz, 1H); ^{13}C-NMR (100 MHz, CD_3OD, **4** MeOH adduct) δ 159.5, 159.1, 156.1, 150.3, 149.9, 138.7, 138.6, 134.3, 131.2, 130.8, 130.4, 128.6, 128.5, 127.9, 127.2, 125.2, 124.7, 124.3, 120.4, 120.2, 119.0, 115.9, 115.2, 114.9, 112.3, 110.7, 108.6, 107.1, 69.1; HRMS (ESI) calcd for $C_{29}H_{20}N_2O_4Na$ $[(M+MeOH)+Na]^+$ 483.1333, Found 483.1315.

Compound **7a**. A solution of phenyllithium (1.15 M cyclohexane/Et_2O; 0.520 mL, 0.598 mmol) was added dropwise over 4 min to a solution of **5** (100 mg, 0.240 mmol) in dry THF (2.5 mL) under nitrogen at –78 °C. After stirring for 2 h, the reaction mixture was poured into the mixed solvent of EtOAc and 0.5 M aq. HCl. The organic layer was separated and washed sequentially with water and brine, before being dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The resulting residue was purified by column chromatography (SiO_2; *n*-hexane/EtOAc = 8:1) to afford compound **7a** (107.8 mg, 91% yield) as a white powder.; mp 71-73 °C, IR (KBr) 3494, 2927, 2852, 1631, 1498, 1423, 1392, 1360, 1317, 1267, 1157, 1007, 885 cm^{-1}; ^{1}H-NMR; (400 MHz, $CDCl_3$) δ 7.94 (s, 2H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.52 (s, 2H), 7.42 (d, *J* = 7.2 Hz, 2H), 7.35 (d, *J* = 2.4 Hz, 2H), 7.24 (dt, *J* = 7.2 Hz, 1.6 Hz, 2H), 7.16 (dt, *J* = 6.6 Hz, 1.2 Hz, 1H), 7.08 (dd, *J* = 9.2 Hz, 2.4 Hz, 2H), 5.28 (s, 2H), 5.27 (s, 2H), 3.52 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.8, 155.8, 149.4, 149.4, 135.0, 135.0, 129.7, 129.7, 128.3, 128.3, 128.1, 127.3, 127.3, 126.9, 126.9, 126.3, 125.9, 125.9, 117.9, 117.9, 111.1, 111.1, 108.9, 108.9, 94.4, 94.4, 71.6, 56.1, 56.1; HRMS (EI) calcd for $C_{31}H_{26}O_6Na$ $(M+Na)^+$ 517.16237, Found 517.16216.

Compound **7b**. A solution of *n*-BuLi (1.60 M *n*-hexane solution; 0.186 mL, 0.576 mmol,) was added dropwise over 5 min to a solution of **6b** (69.3 mg, 0.576 mmol,) in dry THF (3 mL) under nitrogen at –78 °C. After stirring for 2.5 h, a solution of **5** (80.0 mg, 0.192 mmol) in dry THF (2.0 mL) was added dropwise to the reaction mixture, and the solution was stirred at –78 °C for 2.3 h. The reaction solution was poured into the EtOAc and water. The organic layer was separated and washed successively with 1 M aq. HCl, water and brine, before being dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The resulting residue was purified by column chromatography (SiO_2; *n*-hexane/EtOAc = 4:1) to afford compound **7b** (63.4 mg, 65% yield) as pale green oil.; IR ($CHCl_3$) 3444, 2956, 2900, 1631, 1498, 1423, 1389, 1263, 1146, 1076, 1001, 887, 776 cm^{-1}; ^1H-NMR; (400 MHz, $CDCl_3$) δ 8.56 (dd, *J* = 8.0 Hz, 1.2 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 2H), 7.51 (s, 4H), 7.45 (dt, *J* = 5.6 Hz, 2.8 Hz, 1H), 7.35 (d, *J* = 2.8 Hz, 2H), 7.25 (dt, *J* = 7.4 Hz, 1.6 Hz, 1H), 7.04 (dd, *J* = 8.8 Hz, 2.4 Hz,2H), 6.93 (d, *J* = 7.2 Hz, 1H), 5.29-5.25 (m, 4H), 3.52 (s, 3H), 3.51 (s, 3H), 1.37 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.9, 155.9, 149.2, 149.2, 143.7, 135.8, 135.2, 135.2, 132.0, 129.7, 129.7, 128.0, 128.0, 127.9, 126.2, 126.2, 126.1, 126.0, 126.0, 125.3, 117.8, 117.8, 111.0, 111.0, 108.3, 108.3, 94.4, 94.4, 71.0, 56.1, 56.1, 21.2; HRMS (EI) calcd for $C_{32}H_{28}O_6Na$ $(M+Na)^+$ 531.17790, Found 531.17781.

Compound **8c**. A solution of *n*-BuLi (1.60 M *n*-hexane solution; 0.229 mL, 0.366 mmol) was added dropwise over 5 min to a solution of **6c** (48.0 mL, 0.360 mmol) in dry THF (3 mL) under nitrogen at –78 °C. After stirring for 2 h, a solution of **5** (50.0 mg, 0.120 mmol) in dry THF (1.0 mL) was added dropwise to the solution, and the resulting mixture was stirred at –78 °C for 2 h. A solution of 4 M hydrogen chloride in 1,4-dioxane was added to the reaction mixture and stirred for 1 min at room temperature. The reaction mixture was poured into EtOAc and water. The organic layer was separated and washed sequentially with water (twice) and brine, before being dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The resulting residue was purified by column chromatography (SiO_2; *n*-hexane/EtOAc = 3:1~2:1) to afford compound **8c** (24.8 mg, 50% yield) as purple solid.; mp 154-155 °C IR (KBr) 3433, 2939, 1620, 1564, 1533, 1469, 1385, 1294, 1201, 1186, 1151, 1122, 1078, 993 cm^{-1}; ^1H-NMR; (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 9.2 Hz, 1H), 7.54 (s, 1H), 7.43 (t, *J* = 7.2 Hz, 1H), 7.34 (d, *J* = 2.4 Hz, 1H), 7.30 (d, *J* = 1.6 Hz, 1H), 7.29 (d, *J* = 2.4 Hz, 2H), 7.22 (d, *J* = 9.6 Hz, 1H), 7.09 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 6.87 (s,1H), 6.73 (s, 1H), 6.54 (dd, *J* = 9.6 Hz, 1.6 Hz, 1H), 6.48 (s,1H), 5.32 (s, 2H), 3.52 (s, 3H), 2.06 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 186.2, 157.7, 150.4, 149.6, 146.7, 142.4, 138.2, 137.8, 136.7, 136.2, 132.4, 130.8, 130.7, 129.9, 129.2, 128.1, 128.0, 127.7, 126.6, 126.4, 123.17, 119.9, 119.1, 118.9, 110.8, 108.5, 105.2, 94.3, 56.3, 23.8, 19.9; HRMS (EI) calcd for $C_{31}H_{24}O_4Na$ $(M+Na)^+$ 483.15747, Found 483.15668.

Compound **9a**. A solution of trifluoroacetic acid (0.30 mL) was added dropwise to a solution of **7a** (9.7 mg, 0.0196 mmol) in CH_2Cl_2 (0.30 mL) and stirred for 2 h at room temperature. To the reaction mixture, toluene (1.0 mL) was added and the reaction mixture was evaporated in vacuo. The resulting residue was purified by column chromatography (SiO_2; *n*-hexane/EtOAc = 4:1) to afford compound **9a** (5.4 mg, 71% yield) as a deep blue powder.; mp >300 °C; IR (KBr) 3431, 3398, 1614, 1597, 1481, 1439, 1377, 1252, 1188, 1126, 962, 876, 847, 806, 777, 704 cm^{-1}; ^1H-NMR; (400 MHz, CD_3OD) δ 7.64 (s, 2H), 7.57 (d, *J* = 9.2 Hz, 2H), 7.42 (s, 2H), 7.41 (dd, *J* = 8.4 Hz, 0.8 Hz, 2H), 7.23 (dt, *J* = 6.8 Hz, 1.2 Hz, 2H), 7.12 (dt, *J* = 6.8 Hz, 2.0 Hz,1.2 Hz, 1H), 7.06 (d, *J* = 2.4 Hz, 2H), 6.92 (dd, *J* = 9.2 Hz, 2.4 Hz, 2H); (100 MHz, CD_3OD, **9a**

MeOH-d_4 adduct) δ 157.6, 151.9, 151.0, 137.2, 130.7, 130.3, 128.9, 127.6, 127.3, 126.8, 123.6, 118.6, 110.6, 108.3, 78.1; HRMS (EI) calcd for $C_{28}H_{20}O_4Na$ [(M+MeOH)+Na]$^+$ 443.12538, Found 443.12538.

Compound **9b**. A solution of 4 M hydrogen chloride in 1,4-dioxane (0.21 mL) was added dropwise to a solution of **7b** (20.0 mg, 0.039 mmol) in 1,4-dioxane (0.20 mL) and stirred for 5 h at room temperature. The reaction mixture was evaporated in vacuo, *n*-hexane was added and the precipitate was collected by filtration to afford compound **9b** (10.9 mg, 69%) as a blue powder.; mp >300 °C; IR (KBr) 3433, 3398, 2920, 2806, 1595, 1481, 1433, 1400, 1371, 1254, 1188, 1128, 964, 904 cm-^{1}; ^{1}H-NMR; (400 MHz, CD_3OD) δ 8.29 (s, 2H), 8.05 (d, *J* = 8.8 Hz, 2H), 8.05 (s, 2H), 7.75 (dt, *J* = 6.8 Hz, 1.2 Hz, 1H), 6.14 (t, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 6.8 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 2.0 Hz, 2H), 7.23 (dd, *J* = 8.8 Hz, 2.4 Hz, 2H), 2.11 (s, 3H); 13C-NMR (100 MHz, CD_3OD+NaOD/D_2O,+H_2O) δ 167.6, 152.2, 147.5, 138.9, 137.1, 132.8, 129.7, 129.5, 128.1, 126.8, 125.9, 124.7, 124.4, 119.1, 109.9, 107.9, 77.0, 21.2 ; HRMS (EI) calcd for $C_{28}H_{18}O_3Na$ (M+Na)$^+$ 425.11470, Found 425.11482.

Compound **9c**. A solution of 4 M hydrogen chloride in 1,4-dioxane (0.20 mL) was added dropwise to a solution of **8c** (13.2 mg, 0.028 mmol) in 1,4-dioxane (0.20 mL) and stirred for 1 h at room temperature. The reaction mixture was evaporated in vacuo, *n*-hexane was added to the residue and precipitate was collected by filtration to afford compound **9c** (6.4 mg, 55%) as a purple powder.; mp >300 °C; IR (KBr) 3398, 2960, 1616, 1595, 1483, 1431, 1254, 1188, 1130, 996, 900, 876, 768 cm^{-1}; ^{1}H-NMR; (400 MHz, CD_3OD) δ 8.27 (s, 2H), 8.13 (s, 2H), 8.07 (d, *J* = 9.2 Hz, 2H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.47 (d, *J* = 7.6 Hz, 2H), 7.37 (d, *J* = 2.0 Hz, 2H), 7.26 (dd, *J* = 9.2 Hz, 2.0 Hz, 2H), 2.00 (s, 6H); ^{13}C-NMR (100 MHz, CD_3OD+NaOD/D_2O) δ 180.8, 161.3, 151.9, 145.5, 137.5, 135.1, 132.3, 130.7, 129.0, 128.6, 127.4, 118.5, 113.1, 106.8, 19.9 (one peak overlapped); HRMS (FT-ICR-MS) calcd for $C_{29}H_{20}O_3Na$ (M+Na)$^+$, 439.13148; Found, 439.13047.

ACKNOWLEDGEMENTS

The authors are grateful to Ms. Akiko Fujihashi (ICR, Kyoto University) for the HRMS measurements, Ms. Kyohko Ohmine (ICR, Kyoto University) for the NMR measurements, and Iain Mackie, PhD, for editing a draft of this manuscript. This study was supported in part by Japan Society for the Promotion of Science (JSPS) KAKENHI (Nos. 26293005 and 19H03355) and Japan Science and Technology Agency A-STEP (JPMJTM19YQ). This study was carried out using the Fourier transform ion cyclotron resonance mass spectrometer and the nuclear magnetic resonance spectrometer (NMR) in the Joint Usage/ Research Center at the Institute for Chemical Research, Kyoto University.

REFERENCES AND NOTES

1. A. Baeyer, *Ber. Dtsch. Chem. Ges.*, 1871, **4**, 555.

2. (a) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515; (b) A. Loudet and K. Burgess, *Chem. Rev.*, 2007, **107**, 4891; (c) K. Kikuchi, *Chem. Soc. Rev.*, 2010, **39**, 2048; (d) H. Kobayashi, M. Ogawa, R. Alford, P. L. Choyke, and Y. Urano, *Chem. Rev.*, 2010, **110**, 2620; (e) R. N. Dsouza, U. Pischel, and W. M. Nau, *Chem. Rev.*, 2011, **111**, 7941; (f) L. Yuan, W. Lin, K. Zheng, L. He, and W. Huang, *Chem. Soc. Rev.*, 2013, **42**, 622; (g) X. Chen, F. Wang, J. Y. Hyun, T. Wei, J. Qiang, X. Ren, I. Shin, and J. Yoon, *Chem. Soc. Rev.*, 2016, **45**, 2976; (h) T. Egawa, Y. Koide, K. Hanaoka, T. Komatsu, T. Terai, and T. Nagano, *Chem. Commum.*, 2011, **47**, 4162; (i) A. Fukunaga, S. Suda, M. Taki, E. Yamaguchi, M. Grzybowski, Y. Sato, T. Higashiyama, and S. Yamaguchi, *Chem. Commun*, 2016, **52**, 1120.
3. M. Sibrian-Vazquez, J. O. Escobedo, M. Lowey, F. R. Fronczek, and R. M. Strongin, *J. Am. Chem. Soc.*, 2012, **134**, 10502.
4. Y. Yang, M. Lowry, X. Xu, J. O. Escobedo, M. Sibrian-Vazpuenz, L. Wang. C. M. Schowalter, T. J. Jensen, F. R. Fronczek, I. M. Warner, and R. M. Strongin, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, **105**, 8829.
5. E. Azuma, N. Nakamura, K. Kuramochi, T. Sasamori, N. Tokitoh, I. Sagami, and K. Tsubaki, *J. Org. Chem.*, 2012, **77**, 3492.
6. H. Yamashita, C. Minari, E. Azuma, K. Kuramochi, A. Imayoshi, and K. Tsubaki, *Heterocycles*, 2020, **101**, 536.
7. Y. Urano, M. Kamiya, K. Kanda, T. Ueno, K. Hirose, and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 4888.
8. A. Yamagami, K. Kawano, S. Futaki, K. Kuramochi, and K. Tsubaki, *Tetrahedron*, 2017, **73**, 7061.
9. A. Yamagami, H. Ishimura, A. Katori, K. Kuramochi, and K. Tsubaki, *Org. Biomol. Chem.*, 2016, **14**, 10963.
10. R. Hirata, A. Torii, K. Kawano, S. Futaki, A. Imayoshi, and K. Tsubaki, *Tetrahedron*, 2018, **74**, 3608.
11. Gaussian 09, Revision D.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli,

J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

12. The optical properties of L-shaped dibenzoxanthene with 2,6-dimenthylphenyl substituent (**12**) are shown in Supporting Information.
13. Y. Yang J. O. Escobedo, A. Wong, C. M. Schowalter, M. C. Touchy, L. Jiao, W. E. Crowe, F. R. Fronczek, and R. M. Strongin, *J. Org. Chem.*, 2005, **70**, 6907.
14. K. Sezukuri, M. Suzuki, H. Hayashi, D. Kuzuhara, N. Aratani, and H. Yamada, *Chem. Commum.*, 2016, **52**, 4872.
15. R. Reisfeld, *J. Phys. Colloq*, 1987, **48**, C7-423.