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ARTICLE

Synthesis and evaluation of a new water-soluble fluorescent red dye, xanthene bis-*C*-glycoside

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Abstract

Condensation of 2 eq. of *C*-*β*-D-glucosylphloroacetophenone with glyoxylic acid in an aqueous solution of Na_2CO_3, followed by air oxidation in MeOH in the presence of 11 eq. of pyridine to afford a 36% yield of a bright red dye, xanthene bis-*C*-glycoside. This dye is 10 times more fluorescent ($\Phi_{f\ [EtOH]}^{581\ nm} = 3.9 \times 10^{-2}$) and 7.5 times more water-soluble (57 mg/mL H_2O) than the natural red pigment, carthamin. Detailed NMR analysis of its methyl analogs was used to confirm the structure of the dye as methyl 4,5-diacetyl-1,3,8-trihydroxy-3-oxo-3*H*-2,7-di-*C*-*β*-D-glucopyranosylxanthene-9-carboxylate from among three possible ring-closure isomers. Xanthene is safe and shows high light-resistance; therefore, xanthene bis-*C*-glycoside could be used as a food colorant or an in vivo probe.

1 | INTRODUCTION

Many red-colored extracts from insects, metabolites of microorganisms, or plants, which contain carminic acid, monascorubin, betanin, lycopene, carthamin, and so on, as the main components, have been used as natural red food colorants for enhancing the aesthetics of foods and the appetite of the consumer. However, some of these natural resources are not only limited and expensive, but also exhibit low stability, low water solubility, and are toxic.[1,2]

Xanthene, the moiety responsible for the fluorescence of compounds like fluorescein, is known as a safe and highly fluorescent dye, and therefore, many fluorescein analogs have been developed to be applied as in vivo probes.[3–5]

We synthesized a water-soluble, bright red fluorescent dye, xanthene bis-*C*-glycoside **1** from *C*-glucosylphloroacetophenone **2**[6,7] under mild conditions without the use of harmful reagents. The two-step synthetic method is simple and environment-friendly. This article aims to describe the synthetic method, structural analysis, and evaluation of these red dyes as candidates for food colorants and in vivo probes.

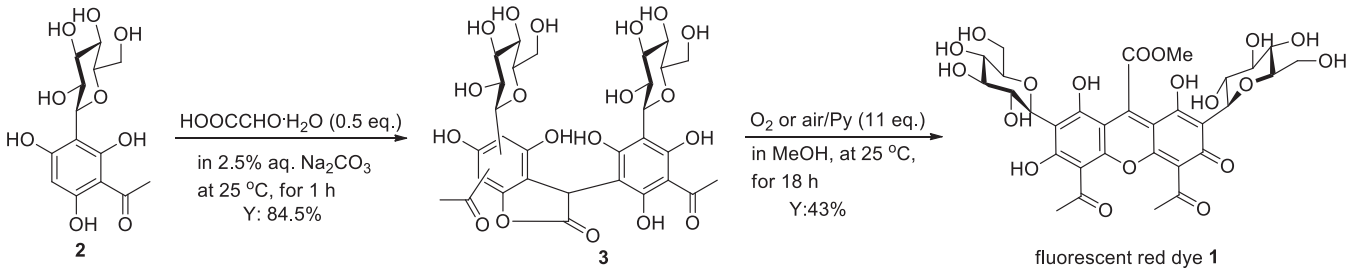
2 | RESULTS AND DISCUSSION

The starting material for the synthesis of **1**, 3-*C*-*β*-D-glucopyranosylphloroacetophenone (**2**) was readily prepared in 48% yield by direct *C*-glycosylation achieved by refluxing phloroacetophenone and D-glucose in CH_3CN-H_2O in the presence of a catalytic amount of $Sc(OTf)_3$.[6,7] This reaction was rendered safer and cheaper by warming at 40°C to 60°C in an aqueous solution of $NaHCO_3$ or Na_2CO_3, but resulted in lower yield (20%-25%). Condensation of 2 eq. of **2** with glyoxylic acid in aqueous Na_2CO_3 solution proceeded smoothly to give an 84.5% yield of **3**. The condensation reaction resulted in two isomers due to the formation of a γ-lactone formed by the condensation between a carboxylic acid and an *ortho*-positioned phenol-hydroxyl group of phloroacetophenone. Due to its inseparable and unstable nature, **3** was subjected to the next oxidation step without further purification. A solution of **3** in MeOH in the presence of 11 eq. of pyridine and O_2 or air was stirred for 18 hours at 25°C.[8] The reaction mixture, which had turned reddish violet in color, was passed through a silica-gel column following which the bright red fluorescent dye **1** was obtained with a 43% yield (Scheme 1).

Since **1** is a red fluorescent dye and the ^{1}H NMR of its methyl analogs (vide infra) showed a characteristic signal at 18 ppm (Figures S9 and S11), it was assumed that the oxidation of **3** was followed by a ring closure to afford a xanthene ring. However, the ring-closure can result in three possible structural isomers, A, B, and C, which are also present in **1**. Their structures of the isomers cannot be determined by direct NMR analysis because of the rotamer due to the two *C*-glycoside moieties and tautomerism between the benzene rings of the xanthene moiety (Figure 1). Therefore, analogs of **1** were synthesized, in which two glucose residues are replaced with methyl groups (Scheme 2).

Condensation of 3-methylphloroacetophenone **4**[9] with glyoxylic acid following a method similar to the synthesis of **1** resulted in a 94% yield of γ-lactone **5**, which was easily crystallized. The solution of **5** could be readily oxidized. Similar to **3**, the dimeric γ-lactone **5** was subjected to the next oxidation step without further purification to afford two main dyes: dye **6** was a reddish-purple nonfluorescent dye (50% yield), while dye **7** (36% yield) was fluorescent and was red in color like **1** (Figure 2). The ^{1}H and ^{13}C NMR spectra of **6** matched those predicted for the asymmetrical isomer C (Figures S9 and S10). On the contrary, NMR spectra of **7** were similar to those predicted for the symmetrical isomers, A or B (Figures S11 and S12). Moreover, the NMR spectra of **1** were also analogous to those of **7** (Figures S3 and S4). The above results indicate that **1** and **7** have a symmetrical structure like A or B, and **6** has an asymmetrical structure, C. Assignment of the xanthene skeleton was done by comparing the recorded ^{13}C NMR spectrum to that NMR spectrum predicted by Chem Draw and to those of the other xanthenes[10,11] and *C*-glycosylphloroacetophenones.[6,7,12]

Xanthene acetates **6′** and **7′** were obtained by acetylation of **6** and **7**, respectively. Both compounds are characterized by two methylphloroacetophenones generated by the introduction of an acetoxy group to the sp^{2} carbon, 9C, of the benzoquinone ring. They both appeared colorless and presented no tautomerism. **6′** and **7′** differ in



SCHEME 1 Synthesis of the fluorescent red-dye xanthene di-C-glycoside **1**

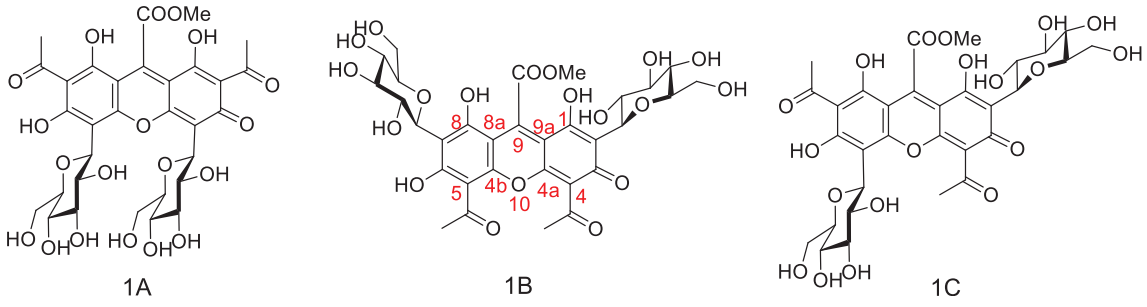
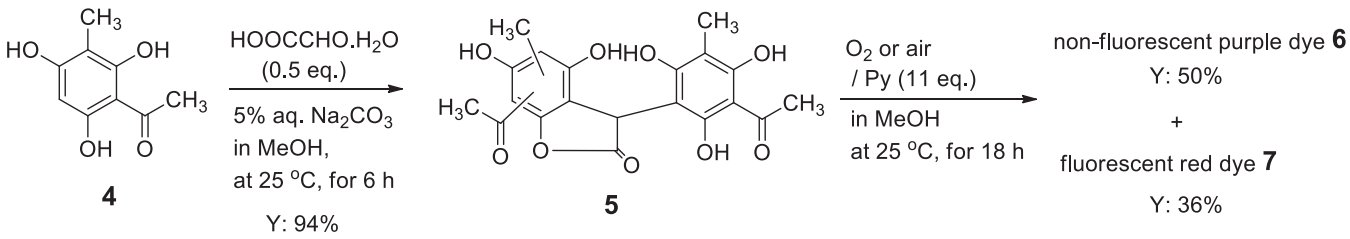


FIGURE 1 Three possible ring-closure isomers, (A, B, and C), of xanthene **1**



SCHEME 2 Synthesis of the xanthene dyes with methylphloroacetophenone **4**

positions of the methyl and acetyl groups (Figures 3, 4, and S9-S12). The ^{1}H NMR spectra of **6′** and **7′** were simpler than those of **6** and **7**. ^{1}H NMR analysis of **6′** showed two acetyl, two methyl, and four *O*-acetyl groups, while the analysis of **7′** showed one acetyl, one methyl, and two *O*-acetyl groups. The ^{1}H NMR spectrum of the acetate of **1** (**1′**) was also similar to that of **7′**, even though multiple signals are observed due to the rotamer of the *C*-glycoside (Figures S5 and S6). Heteronuclear multiple bond coherence (HMBC) spectrum of **7′** showed correlations between methyl protons (1.78 ppm) and 1C (147.29 ppm), 2C (117.52 ppm), and 3C (150.55 ppm), and a correlation between the acetyl protons (2.39 ppm) and 4C (124.71 ppm) (Figures 4 and S18). HMBC analysis of **7** also showed correlations between methyl protons (2.00 ppm) and 1C (157.63 ppm), 2C (98.17 ppm), and 3C-carbonyl (176.2 ppm) (Figures 5 and S17). Based on these results, **7** and **7′** were assigned the structure B, wherein C2 and C7 are methyl groups, and C4 and C5 are acetyl groups.

The HMBC analysis of **1′** also showed correlations between glucosyl anomeric protons (5.48 and 5.49 ppm) and 1C (149.4 and 149.5 ppm), 2C (114.63 and 114.91 ppm), and 3C (148.3 and 148.7 ppm), and correlations between acetyl protons (2.38 and 2.40 ppm) and 4C (126.52 and 127.35 ppm) (Figure 6). The HMBC spectrum of **1** showed correlations between H1′ and H1″ (4.59 and 4.62 ppm) of the glucose residue and 2C and 7C (108.64, 109.02, and 109.17 ppm). **1** was also determined to be isomer B.

The UV-Vis spectrum of **1** in EtOH showed an absorption maximum at 537 nm (log ε = 4.29), making **1** appear purple-red in color. The absorbance of **1** is higher than that of carthamin (Figure 7) [λ_{max}^{EtOH} (log ε) 518 (4.07) nm]. The fluorescence quantum yields were obtained from the following equation:

$$\Phi_{sample} = \Phi_{standard} \times \left(A_{standard}/A_{sample}\right) \times \left(\Sigma\left[F_{sample}\right]/\Sigma[F_{standard}]\right) \times \left(n^2_{sample}/n^2_{standard}\right) \quad (1)$$

where A, F, Σ [F], and n^2 denote the absorbance, fluorescence intensity, the peak area of the fluorescence spectra, and refractive index of the used solvent, respectively.

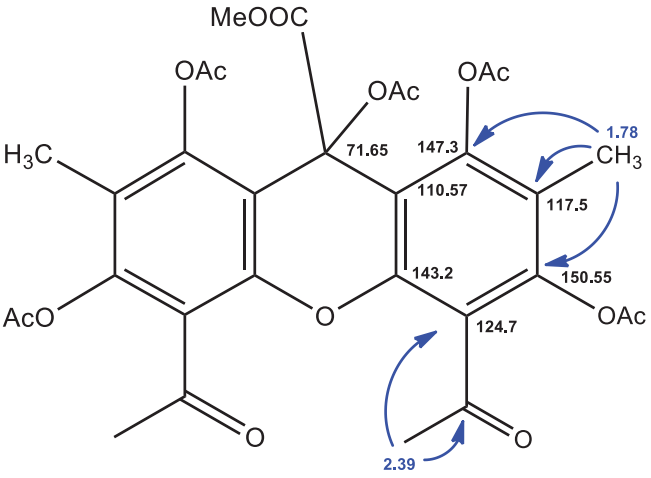


FIGURE 4 Heteronuclear multiple bond coherence correlation of **7′**

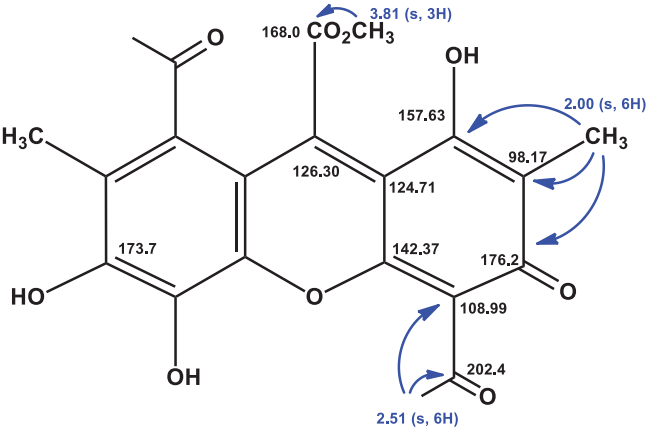


FIGURE 5 Heteronuclear multiple bond coherence correlation of **7**

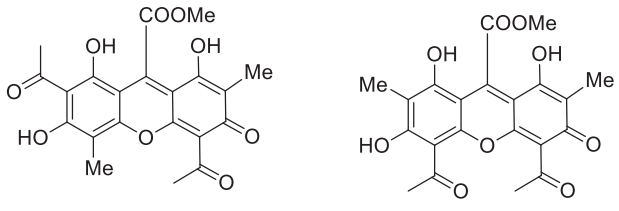
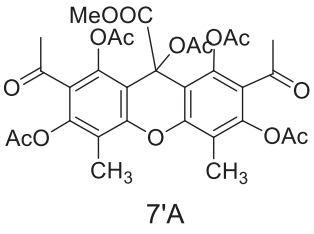
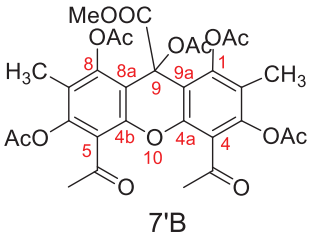


FIGURE 2 Structure of the methyl analogs **6** and **7**





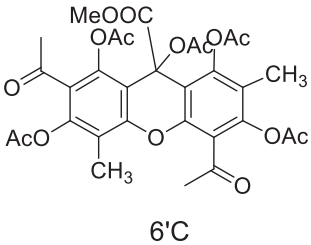


FIGURE 3 The structures of **6′** and **7′**

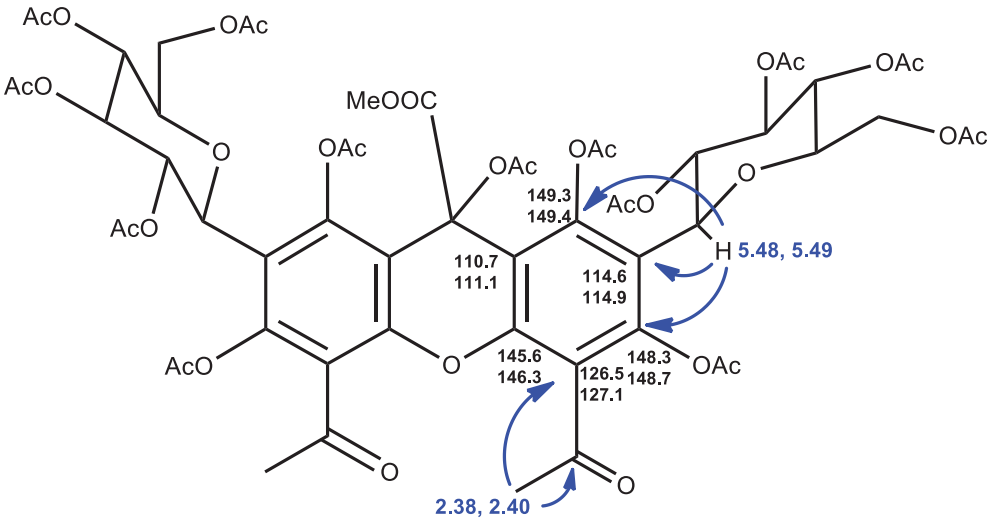


FIGURE 6 Heteronuclear multiple bond coherence correlation of **1′**

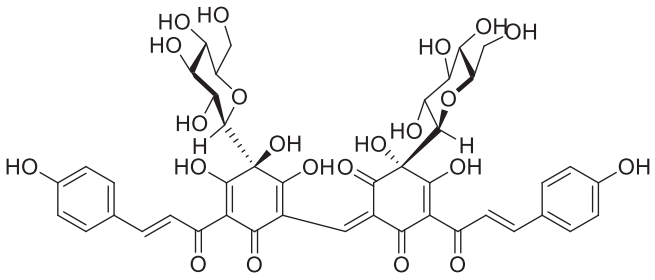


FIGURE 7 Red pigment, carthamin

Here, anthracene was as a standard and its quantum yield ($\Phi_{standard}$) in EtOH is 0.27.[13]

The fluorescence quantum yields of **1** ($\Phi_{f\ (EtOH)}^{581\ nm} = 3.9 \times 10^{-2}$) and of its methyl analog **7** ($\Phi_{f\ (EtOH)}^{606\ nm} = 2.5 \times 10^{-2}$) are considered low for a fluorescent agent; however, they are suitable for **1** to be used as a food colorant and are 10 times higher than that of carthamin ($\Phi_{f\ (EtOH)}^{564\ nm} = 3.8 \times 10^{-3}$).[14] Water solubility of **1** (57 mg/mL H_2O) was found to be 7.5 times higher than that of carthamin (7.6 mg/mL H_2O).

3 | CONCLUSION

A fluorescent red-dye, xanthene bis-*C*-glycoside was synthesized with a 36% yield using an environment friendly, two-step reaction with phloroacetophenone *C*-glycoside as precursor. The dye was assigned a symmetrical structure (B), from among three possible isomeric structures, using detailed NMR analysis of its methyl analogs. The dye can find application as a red food colorant and an in vivo probe due to its bright red color, fluorescence, stability, water-solubility, and low toxicity, while the evaluation of its biological activity and toxicity is required.

4 | EXPERIMENTAL

4.1 | General

All solvents and reagents used in this work are commercially available, and utilized without further purification. The reaction progress was monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates with a fluorescent indicator, F254 (Merck). A 5% ferric chloride solution in ethanol, and 7% phosphomolybdic acid solution in ethanol, activated by heat application, were used as the TLC dyeing agents. Separation and purification of the reaction products were performed by flash column chromatography on silica gel 60 (40-50 μm; Kanto Reagents, Japan). Purification of the natural carthamin was performed by Sephadex LH 20 (Merck) gel column chromatography. Melting points were determined using an ASONE micromelting point apparatus, and the melting points so obtained were not corrected. Infrared (IR) spectra were recorded on a Horiba FT-720 IR spectrometer using KBr disks. Fluorescence spectra and relative fluorescence intensity were measured using a Shimadzu RF-6000 fluorescence spectrophotometer. Both excitation and emission wavelength band passes were set at 5 nm and the range 1. Sample concentrations were 29.6 μM for **3** and carthamin, and 22.6 μM for **7**. Absorption spectra were obtained using a Hitachi U-3000 UV-Vis spectrometer. NMR spectra were recorded on ECX-500 and 600 spectrometers (JEOL, Japan). The chemical shifts (δ, ppm) in the ^{1}H NMR spectra are with reference to the proton signal of some partially protonated solvent impurities ($CDCl_3$ at $\delta = 7.26$ ppm, DMSO-d_6 at $\delta = 2.49$ ppm). The carbon signal of $CDCl_3$ ($\delta = 77.0$ ppm) and DMSO-d_6 ($\delta = 39.5$ ppm) were used as reference in the ^{13}C NMR spectra. High-resolution mass spectra were obtained under electrospray ionization conditions on a JMS-T100LP spectrometer (JEOL, Japan).

4.2 | Synthetic method of 3-*C*-*β*-D-glucopyranosylphloroacetophenone (2) by using Na_2CO_3 as a catalyst

Phloroacetohenone (2.48 g, 14.8 mmol) and D-glucose (7.9 g, 43.9 mmol) were dissolved in 0.4 M Na_2CO_3 aq. solution (38 mL), and the stirred mixture in a 200 mL flask equipped with cooler was warmed at 50°C to 55°C for 2 hours. The reaction mixture was cooled and acidified with 2 M HCl (pH 4. Caution! Evolution of CO_2 gas), and the resulting suspension was passed through a column of MCI GEL CHP20P (75-150 μm, Mitsubishi Chemical Corp., 4.0 × 20 cm) loaded with water, and the gel was then washed with 0.6 L of water, to remove nonabsorbed glucose and salts. The absorbed products were eluted from the gel column with 300 mL of 50% aqueous acetone, and the eluate was evaporated in vacuo to give a pale-brown solid (2.5 g) that was then separated by silica-gel column chromatography (15:30:2:1 acetone:EtOAc:H_2O:AcOH) to afford crude **2** (1.3 g), which was recrystallized from EtOH to give **2** (1.15 g, 23.5%) as a white crystal, and recovered phloroacetophenone (0.62 g, 25%).

4.3 | Synthesis of 7-acetyl-3-(3-acetyl-2,4,6-trihydroxy-5-*C*-*β*-D-glucopyranosylphenyl)- and 3-acetyl-7-(3-acetyl-2,4,6-trihydroxy-5-*C*-*β*-D-glucopyranosylphenyl)-4,6-dihydroxy-5-*C*-*β*-D-glucopyranosylbenzofuran-2(3*H*)-one (3)

3-*C*-*β*-D-glucosylphloroacetophenone (**2**) (637 mg, 1.93 mmol) and 0.5 eq. of glyoxylic acid monohydrate (91 mg) were dissolved in 2.5% aq. Na_2CO_3 solution (6.5 mL) and stirred at 25°C under Ar atmosphere for 1 hour. After confirming the complete conversion of **2** into **3** using TLC (acetone:AcOEt:H_2O:AcOH in 30:30:5:1 ratio), Dowex 50W × 8 (H^+) resin was added to the stirred reaction mixture until pH 2 to 3 was obtained; the resulting mixture was filtered and washed with EtOH. The filtrate was evaporated to yield reddish solids that were purified using silica gel column chromatography (acetone:AcOEt:H_2O:AcOH in 15:30:2:1 ratio) to afford **3** (1138 mg, 84.5%) as an ivory colored powder.

Ivory powder. R_f 0.39 (30:30:3:1 acetone:AcOEt:H_2O:AcOH). IR (KBr) ν: 3398, 2924, 1873 (γ-lactone), 1719, 1626, 1439, 1368, 1275, 1046 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.54-2.65 (total 6H, each s, Ac × 2), 3.1-3.4 (4H, m, 3.3 (2H, m, H4′, 4″), 3.4-3.5 (2H, m, H2′, 2″), 3.55-3.75 (2H, m, H6′, 6″), 3.55-3.75 (2H, m, H6′, 6″), 4.67 and 4.90 (each 1H, J = 9.1 Hz, H1′, 1″), 5.32-5.35 (total 1H, each s, >CH), 9.52, 9.71, 9.80, 13.51, 13.52, 13.53, and 13.99 (total 5H, each s, OH × 5). ^{13}C NMR (DMSO-d_6): δ 31.05-31.16, 32.67-33.09, 36.69-37.06, 100.44-100.54, 102.68-103.09, 103.61-104.20, 105.21-105.41, 105.69-106.20, 107.48-107.80, 155.29-155.36, 158.19-158.45, 159.55-160.95, 161.81-162.55, 175.42-175.63, 201.31-201.54, 203.79-204.11. (glucose moiety): 59.48-60.28, 68.59-69.45, 71.95, 72.14, 73.05, 73.21, 74.22, 74.45, 75.28, 75.97, 77.52, 77.72, 78.20, 78.30, 81.0-81.46, 81.83, 81.91. HRESIMS (m/z) Calcd for $C_{30}H_{33}O_{19}$ $[M-H]^-$: 697.1616; Found: 697.1638.

4.4 | Synthesis of methyl 4,5-diacetyl-1,3,8-trihydroxy-3-oxo-3*H*-2,7-bis-*C*-*β*-D-glucopyranosylxanthene-9-carboxylate (1)

The solution of **3** (280 mg, 0.40 mmol) and 11 eq. of pyridine (328 μL) in MeOH (5.6 mL) in a 200 mL flask equipped with cooler or O_2 balloon was vigorously stirred at 25°C for 18 hours. 1 M HCl (1 mL) was added to the red reaction mixture, and the resulting solution was evaporated to give a red solid. The product was purified using silica gel column chromatography (acetone:AcOEt:H_2O:AcOH at 15:30:2:1 to 30:30:5:1 ratio) to afford **1** (122 mg, 43%) as a red crystalline powder.

Red prism (from aqueous acetone). R_f 0.46 (60:45:8:2 acetone:AcOEt:H_2O:AcOH). Mp >300°C. IR (KBr) ν: 3365, 2924, 2893, 1723, 1652, 1577, 1498, 1448, 1434, 1383, 1281 cm^{-1}.

UV-Vis (EtOH) (log ε) 322 (4.39), 356 (sh, 3.87), 537 (4.29) nm. $\Phi_{f\ (EtOH)}^{581\ nm} = 3.9 \times 10^{-2}$. ^1H NMR (DMSO-d_6, 50°C): δ 3.22 (2H, m, H-2′, 2″), 3.22∼3.26 (4H, m, H-3′, 3″ and H-4′, 4″), 3.40 and 3.52 (each 1H, m, H-5′, 5″), 3.65∼3.77 (2H, m, H-6′, 6″), 4.05 and 4.16 (each 1H, m, H-2′, 2″), 4.59 and 4.62 (each 1H, d, J = 10.8 and 10.0 Hz, H-1′, 1″), 3.32, 3.93, and 4.85 (each 1H, br.s, OH × 3), 3.816 (3H, s, OMe), 2.504 and 2.555 (each 3H, s, Ac × 2). ^{13}C NMR (DMSO-d_6, 50°C): δ 203.58, 203.24, 202.74, 174.92, 167.92, 159.46, 159.01, 145.70, 143.10, 127.16 (×2), 109.17, 109.02, 108.64, 100.70, (glucose moiety): 83.03 and 81.88 (C5), 80.61 and 79.84 (C3), 79.40 and 77.74 (C1), 74.33 and 73.03 (C2), 70.64 and 69.94 (C4), 61.99 and 61.50 (C6). HRESIMS (m/z) Calcd for $C_{31}H_{33}O_{19}$ $[M-H]^-$: 709.1616. Found: 709.1639.

4.5 | Synthesis of methyl 1,3,6,8,9-pentaacetoxy-4,5-diacetyl-2,7-bis-*C*-*β*-D-(per-*O*-acetylglucopyranosyl)-9*H*-xanthene-9-carboxylate (1′)

Red xanthene **1** (36 mg, 0.051 mmol) was dissolved in acetic anhydride (0.7 mL) and pyridine (0.3 mL). N,N-dimethyl-4-aminopyridine (DMAP) (10 mg) was added to

the mixture that was then stirred at 25°C for 16 hours. The reaction mixture was extracted twice with AcOEt. The organic layer was washed with 0.1 M HCl and brine, and dried with anhydrous Na_2CO_3. After evaporation, the residue was purified using silica gel column chromatography (1:1 1-hexane:AcOEt) to afford **1′** (42 mg, 64% yield) as an ivory colored powder.

Ivory powder. R_f 0.42 (20:1 $CHCl_3$:MeOH). IR (KBr) ν: 3021, 2940, 2858, 1784, 1754, 1706, 1623, 1594, 1435, 1371, 1229, 1192, 1140, 1112 cm^{-1}.^{1}H NMR (DMSO-d_6): δ 1.617, 1.766, 1.896, 2.011, 2.036, 2.046, and 2.067 (each 3H, s, OAc × 7), 2.290 (6H, s, ArOAc×2), 2.283 and 2.367 (each 3H, s, ArOAc×2), 2.379 and 2.397 (each 3H, s, C-Ac × 2), 3.712 (3H, s, CO_2Me), 3.92 (each 1H, dd, J = 1.6 and 12.5 Hz, H-6′a) and 3.99 (each 1H, dd, J = 1.7 and 13.3 Hz, H-6″a), 4.12 (m, 2H, H5′, 5″), 4.55 (dd, 1H, J = 4.1 and 13.3 Hz, H6′b), 4.68 (dd, 1H, J = 5.0 and 12.5 Hz, H6″b), 5.42 and 5.47 (each t, 1H, J = 9.2 Hz, H3′, 3″), 5.48 and 5.49 (each d, 1H, J = 9.2 Hz, H1′, 1″), 5.52 and 5.57 (each t, J = 9.2 Hz, H2′, 2″). ^{13}C NMR ($CDCl_3$): δ (xanthene moiety) 53.92, 70.79, 114.63, 114.90, 126.52, 127.14, 145.57, 146.32, 148.3, 148.7, 149.3, 149.4, 166.4, 196.34, 196.65, (glucose moiety) 61.94, 62.02, 67.66, 67.72, 71.35, 71.47, 72.56, 72.90, 74.52, 74.61, 77.00, 77.26, (acetyl group) 19.98, 20.24, 20.44, 20.53, 20.56, 20.59, 20.71, 21.08, 21.14, 30.25, 30.58, 167.71, 167.93, 168.02, 168.55, 168.58, 168.79, 169.54, 169.57, 169.78, 169.92, 170.30, 170.37, 196.34, 196.65. HRESIMS: (m/z) Calcd for $C_{57}H_{62}NaO_{33}$: 1297.3071. Found: 1297.3061.

4.6 | Synthesis of 7-acetyl-3-(3-acetyl-2,4,6-trihydroxy-5-methylphenyl)-4,6-dihydroxy-5-methylbenzofuran-2(3*H*)-one and 5-acetyl-3-(3-acetyl-2,4,6-trihydroxy-5-methylphenyl)-4,6-dihydroxy-7-methylbenzofuran-2(3*H*)-one (5)

3-Methylphloroacetophenone (**4**) (119 mg, 0.705 mmol) and 0.5 eq. of glyoxylic acid monohydrate (30 mg, 0.352 mmol) were dissolved in 5% aq. Na_2CO_3 (0.5 mL) and MeOH (1.5 mL); the resulting mixture was stirred at 25°C under Ar atmosphere for 6 hours. After confirming the completion of reaction by means of TLC (toluene: AcOEt:AcOH in 5:2:0.5 ratio), 1 M HCl (3 mL) was added to the mixture, and then extracted twice with AcOEt. The organic layer was washed with water and brine, and then dried with Na_2SO_4. After removing the organic solvent, the residue was purified using silica gel column chromatography (toluene:AcOEt:AcOH with ranging from 6:1:0.2 to 6:3:1) to afford dimer-lactone **5** (266 mg, 94% yield) as a pale-red powder, that was crystallized from EtOH.

Ivory prism (EtOH). Mp > 300°C. R_f 0.41 (5:2:0.5 toluene: AcOEt: AcOH). IR (KBr) ν: 3588, 3470, 3338, 1822, 1617, 1587, 1428, 1406, 1365, 1328, 1296, 1226, 1177 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 13.43, 13.43, 13.10, 11.19, 9.93 (total 5H, each s, OH x 5), 5.38 and 5.35 (total 1H, each s, >CH-), 2.68 and 2.64 (total 6H, s, Ac × 2), 1.883 and 1.877 (total 6H, each s, CH_3 × 2). ^{13}C NMR (DMSO-d_6): δ 7.72, 8.64, 30.64, 32.22, 32.76, 100.36, 102.78, 102.90, 103.12, 105.67, 105.71, 105.78, 105.81, 105.89, 106.02, 153.40, 153.44, 157.54, 157.67, 159.81, 159.99, 160.89, 161.19, 175.78, 175.93, 200.75, 200.93, 203.51, 203.69. HRESIMS: (m/z) Calcd for $C_{20}H_{17}O_9$ [M-H]$^-$: 401.0878. Found: 401.0889.

4.7 | Synthesis of methyl 2,5-diacetyl-1,3,8-trihydroxy-4,7-dimethyl-3-oxo-3*H*-xanthene-9-carboxylate (6) and methyl 2,7-diacetyl-1,6,8-trihydroxy-4,5-dimethyl-3-oxo-3*H*-xanthene-9-carboxylate (7)

Dimer-lactone **5** (197 mg, 0.49 mmol) was dissolved in MeOH (1.0 mL), followed by the addition of the pyridine mixture (11 Equation 436 μL). The resulting solution was stirred vigorously at 25°C under air or O_2 atmosphere (cooler or balloon) for 18 hours. After confirmation of the completion of reaction and the production of two red dyes, the organic solvent was removed by evaporation. The residue was dissolved in AcOEt, washed with a 0.1 M HCl aqueous solution, water, and brine, and then dried with Na_2SO_4. After evaporating the organic solvent, the residue was separated using silica-gel column chromatography (toluene:AcOEt:AcOH with ranging from 6:1:0.2 to 6:3:1 ratio) to afford **6** (102 mg, 50% yield) as a reddish-purple dye and **7** (73 mg, 36% yield) as a fluorescent red dye.

4.8 | Methyl 2,5-diacetyl-1,3,8-trihydroxy-4,7-dimethyl-3-oxo-3*H*-xanthene-9-carboxylate (6)

Dark purple prism (from acetone). Mp > 300°C. R_f 0.79 (6:3:1 toluene:AcOEt:AcOH). IR (KBr) ν: 3254, 2951, 1754, 1647, 1581, 1508, 1448, 1429, 1404, 1274, 1217, 1161 cm^{-1}. UV-Vis (MeOH) (log ε) 284 (4.16), 338 (4.25), 528 (3.98) nm. ^{1}H NMR (DMSO-d_6, 50°C): δ 2.00 and 2.04 (each s, 3H, CH_3 × 2), 2.49 and 2.56 (each s, 3H, Ac × 2), 3.89 (s, 3H, CO_2CH_3), 8.29, 14.6, and 18.02 (each s, 1H, OH × 3). ^{13}C NMR (DMSO-d_6, 50°C): δ 8.03, 13.92, 29.44, 33.20, 52.11, 103.77, 108.41, 108.60, 108.76, 108.81, 115.22, 141.40, 155.51, 156.00, 159.36, 166.70, 167.85, 175.36, 181.15, 202.27, 202.45.

HRESIMS: (*m/z*) Calcd for $C_{21}H_{17}O_9$ $[M-H]^-$: 413.0873. Found: 413.0869.

4.9 | Methyl 4,5-diacetyl-1,6,8-trihydroxy-2,7-dimethyl-3-oxo-3*H*-xanthene-9-carboxylate (7)

Reddish purple prism (from EtOH). Mp > 300°C. R_f 0.42 (6:3:1 toluene:AcOEt:AcOH). UV-Vis (MeOH) λ (log ε) 284 (4.06), 326 (4.55), 362 (3.93), 553(4.41) nm. UV-Vis (EtOH) λ (log ε) 284 (sh, 4.08), 326 (4.55), 362 (sh, 3.96), 555 (4.45) nm. $\Phi_{f\ (EtOH)}{}^{606\ nm} = 2.5 \times 10^{-2}$. IR (KBr) ν: 1730, 1651, 1581, 1431, 1402, 1387, 1282 cm^{-1}. ^1H NMR (DMSO-d_6, 50°C): δ 2.00 (s, 6H, $CH_3 \times 2$), 2.51 (s, 6H, A × 2), 3.81 (s, 3H, CO_2CH_3), 14.65, and 17.95 (OH × 2). ^{13}C NMR (DMSO-d_6, 50°C): δ 6.83, 30.42, 51.80, 98.10, 108.99, 126.30, 143.70, 157.62, 167.96, 173.73, 176.20, 202.36. HRESIMS (*m/z*) Calcd for $C_{21}H_{17}O_9$ $[M-H]^-$: 413.0873. Found: 413.0867.

4.10 | Synthesis of methyl 1,3,6,8,9-pentaacetoxy-2,5-diacetyl-4,7-dimethyl-9*H*-xanthene-9-carboxylate (6′) and methyl 1,3,6,8,9-pentaacetoxy-4,5-diacetyl-2,7-dimethyl-9*H*-xanthene-9-carboxylate (7′)

Reddish purple xanthene **6** (50 mg, 0.12 mmol) was dissolved in acetic anhydride (1.0 mL) along with pyridine (0.5 mL) and 10 mg of DMAP (10 mg). The mixture was stirred at 25°C for 5 hours and then extracted twice with AcOEt. The organic layer was washed with 0.1 M HCl and brine, and dried with anhydrous Na_2CO_3. After evaporation, the residue was crystallized from AcOEt to afford **6′** (79 mg, 98% yield) as white crystals. Acetate of **7** (**7′**) was also obtained with a 97% yield through a method similar to the acetylation of **6**. (Because the Rf value of **6′** and **7′** was same, they are inseparable after acetylation of the mixture of **6** and **7**).

4.11 | Synthesis of methyl 1,3,6,8,9-pentaacetoxy-2,5-diacetyl-4,7-dimethyl-9*H*-xanthene-9-carboxylate (6′)

White prism (from AcOEt). R_f 0.41 (at 1:1 ratio AcOEt:1-hexane). Mp 224°C-226°C. IR (KBr) ν: 2951, 1773, 1701, 1595, 1432, 1370, 1278, 1226, 1184, 1125 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.80 and 1.90 (each 3H, s, $CH_3 \times 2$), 1.80 (3H, s, OAc), 2.30, 2.31, 2.36, 2.38 (each 3H, s, ArOAc×4), 2.39 and 2.67 (each 3H, s, ArAc×2), 3.76 (3H, s, $COOCH_3$). ^{13}C NMR ($CDCl_3$): δ 9.01, 9.10, 52.75, 70.42, 109.73, 110.02, 116.31, 120.18, 120.63, 123.89, 142.37, 145.70, 146.26, 146.42, 147.04, 149.56, (C-Ac): 29.91 and 30.78, 196.61 and 197.71, (C═O × 6): 19.45, 19.63, 19.68, 19.80, 19.83 (×2), 166.01, 166.90, 167.38, 167.55, 167.61, 168.26. HRESIMS (*m/z*) Calcd for $C_{31}H_{30}NaO_{15}$ $[M-H]^-$: 665.1482. Found: 665.1480.

4.12 | Methyl 1,3,6,8,9-pentaacetoxy-4,5-diacetyl-2,7-dimethyl-9*H*-xanthene-9-carboxylate (7′)

White prism (from AcOEt). R_f 0.41 (at 1:1 ratio AcOEt:1-hexane). Mp 230°C-232°C. IR (KBr) ν: 1773, 1700, 1595, 1370, 1280, 1226, 1188, 1122 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.78 (s, 3H, 9-OAc), 2.25 (6H, s, $CH_3 \times 2$), 2.17 (3H, s, OAc), 2.25 and 2.30 (each 6H, s, ArOAc×4), 2.39 (6H, s, ArAc×2), 3.75 (3H, s, $COOCH_3$). ^{13}C NMR ($CDCl_3$): δ 9.73 (×2), 53.67, 71.65, 110.57, 117.52, 124.71, 143.20, 147.29, 150.55, (C-Ac): 30.82 (× 2), 197.51 (×2), (CO_2CH_3): 53.67, 166.97, (OAc × 5): 20.55 (×2), 20.69 (×3), 167.82, 168.50 (×2), 169.16 (×2). HRESIMS (*m/z*) Calcd for $C_{31}H_{30}NaO_{15}$ $[M-H]^-$: 665.1482. Found: 665.1475.

4.13 | Natural carthamin

Natural carthamin was prepared by purifying Lio Flesh (TOYO ADR, Japan) by silica gel column chromatography (upper layer; 1-BuOH:H_2O:AcOH at 4:5:1 ratio) followed by Sephadex LH-20 gel column chromatography in MeOH. It was used as control for the spectral and water solubility measurements.

4.14 | Water solubility

Fifty microliters of H_2O was added at a time to 5.3 mg of carthamin and the suspension was dissolved by supersonic stirring at 25°C. Addition of water in steps of 50 μL and stirring was repeated until all solid was dissolved. A total 700 μL of H_2O was needed until complete dissolution. For 5.7 mg of **1**, with the addition of 25 μL of H_2O at a time and supersonic stirring, complete dissolution was reached with a total of 100 μL of H_2O.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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