Development of Water Solubility of 2-Phenylsulfanylhydroquinone Dimer Dye

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insoluble in water → 2 water soluble

Fluorescence of **2** in aqueous media

BSA-**2** complex detected
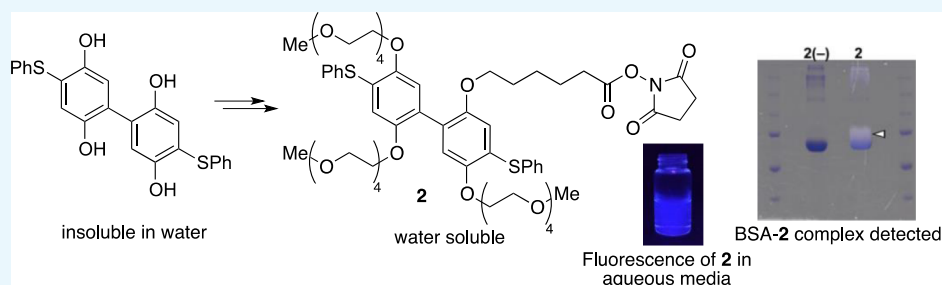
ABSTRACT: With the aim of developing a new fluorescence dye with enhanced photophysical properties, this study describes the modification of the 2-phenylsulfanylhydroquinone dimer to realize a new bioimaging molecule. The characteristics of the dimer were advanced by introducing tetraethylene glycol side chains to provide sufficient water solubility and a tether consisting of an *N*-hydroxysuccinimide-terminated C6-carbon chain to attach bioactive molecules. Two derivatives containing two or three tetraethylene glycol side chains were designed and prepared, and the latter showed sufficient water solubility for biochemical applications. Both compounds exhibited similar photophysical properties and blue fluorescence under UV light irradiation. The dye containing three tetraethylene glycol units reacted with bovine serum albumin in water to give fluorescent derivatives.

■ INTRODUCTION

Organic fluorophores are recognized as important tools to probe chemical and biological reactions.[1] These compounds provide a useful method to trace very tiny amounts of target molecules by monitoring their photoluminescence when attached to a fluorophore. For example, some fluorescence probes have been applied for the detection of formaldehyde[2] or some nerve agents.[3] They are also applied to sense the change of microenvironment such as polarity, viscosity, and molecular assembly.[4] In these cases, target molecules or chemical environments undergo chemical reactions with the fluorescent probes and change the fluorescence properties that we may detect.[5] Such reactions include a wide range of chemical events, covering from acid−base reactions to inclusion phenomena.[6] For example, an oxidative biological reaction *in vivo* was detected and monitored by a rhodamine-modified dye in a real-time manner.[7] Recently, such methodology has been applied to a wide range of cancer detection studies.[8] There are a large variety of fluorophores available for these purposes, including coumarin,[9] fluorescein,[10] phenoxazine,[11] cyanine,[12] and BODIPY.[13] As a wide range of photoluminescence properties are required in order to detect the corresponding analytical or biological events, there still is some space to develop new organofluorophores that have effective physical properties that meet the required analytical purposes. Such properties include from the change of wavelengths of emitssion to the robustness of the dyes in the

actual analytical atmosphere. This is the motivation that attracts synthetic chemists to develop or invent new fluorescent dyes.

We recently developed a new type of fluorescent dye based on the 2-phenylsulfanylhydroquinone dimer,[14] which consists of a biaryl structure that is prepared via oxidative dimerization of hydroquinone dimethylether.[15] This dye showed a relatively strong fluorescence at 403 nm under UV light irradiation of 320 nm, and its quantum yield reached up to 0.3. The preparation of the dye is very convenient, and large-scale synthesis is possible via a facile procedure. The presence of an electron-rich aromatic system enabled the control of fluorescence by the number of acyl groups introduced on the phenolic hydroxyl groups.[16] The advantageous point of the dye is that the dye is expected to be a robust dye against pH and photoexcitation because it is based on a biaryl unit. These properties make the 2-phenylsulfanylhydroquinone dimer-derived dye a promising candidate for the detection and monitoring of chemical and biological events. This compound,
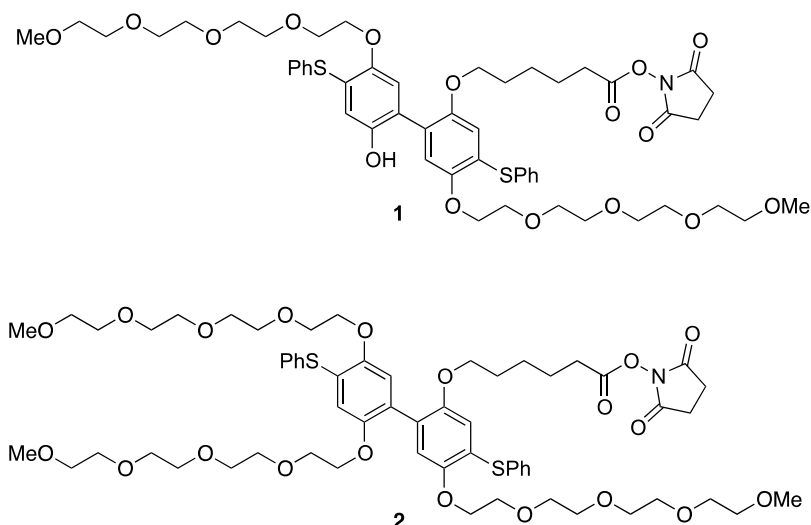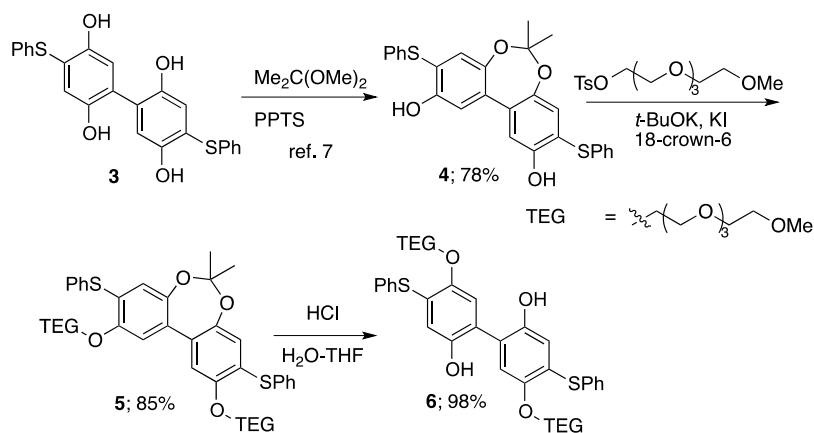
Figure 1. Target molecules of this research.

Scheme 1. Synthesis of Intermediate 6

however, shows poor water solubility, which is essential for organic dyes to be used for this purpose. We were interested in modifying the dye to overcome the solubility issue and to provide a tether site to attach the bioactive molecules, thereby realizing a new bioimaging material. In this paper, we report the modification of the 2-phenylsulfanylhydroquinone dimer for the development of a new bioimaging dye. The application of the dye to attach the bovine serum albumin (BSA) protein in water phase is also demonstrated.

■ RESULTS AND DISCUSSION

The four phenolic hydroxyl groups of the 2-phenylsulfanylhydroquinone dimer were envisaged as reactive to achieve the desired modifications, that is, the introduction of a tether unit to attach a bioactive molecule and the improvement of water solubility. The former modification was approached by a C6-tether containing a carboxylic moiety at its end. Thus, commercially available ethyl 6-bromocapronate was used as the starting material, which was attached to the dimer via a simple S_N2 reaction with one of the phenolic OH groups (Figure 1). Subsequent conversion of the ester unit to an active ester such as N-hydroxysuccinimide (NHS) would facilitate the introduction of a bioactive molecule containing an amino residue.

To improve the water solubility of the dye, we selected the commercially available tetraethylene glycol as a hydrophilic modifier to be attached to the phenolic groups. As we could not anticipate the appropriate number of modifiers to achieve sufficient hydrophilicity when launching the research, we designed compounds **1** and **2**, containing two and three tetraethylene glycol groups, respectively, as the target molecules of the present study (Figure 1).

Our synthesis started from 2-phenylsulfanylhydroquinone dimer dimethyl ketal **4**, which was readily prepared following a reported method from compound **3**.[15] The synthetic outline to the key intermediate **6** is depicted in Scheme 1. Compound **4** was exposed to tetramethylene glycol monomethylether tosylate under basic conditions, giving the 5,5′-glycol derivative **5** in 85% yield. The ketal unit was then removed quantitatively by acidic treatment to give the key intermediate **6**.

To prepare compound **1**, monoprotection of the two phenolic OH groups of **6** was needed. This was achieved efficiently using Boc_2O. Thus, the use of 1 equiv Boc_2O afforded **7** selectively in 92% yield without the formation of the diprotected byproduct. Installation of the tether was readily achieved using 6-bromocapronate ester under standard S_N2 reaction conditions, and compound **8** was isolated in 94% yield. Then, activation of the ethyl ester was achieved by alkaline hydrolysis followed by the reaction with NHS in the

Scheme 2. Synthesis of Compound 1

TEG = -CH₂(CH₂OCH₂)₃CH₂OMe
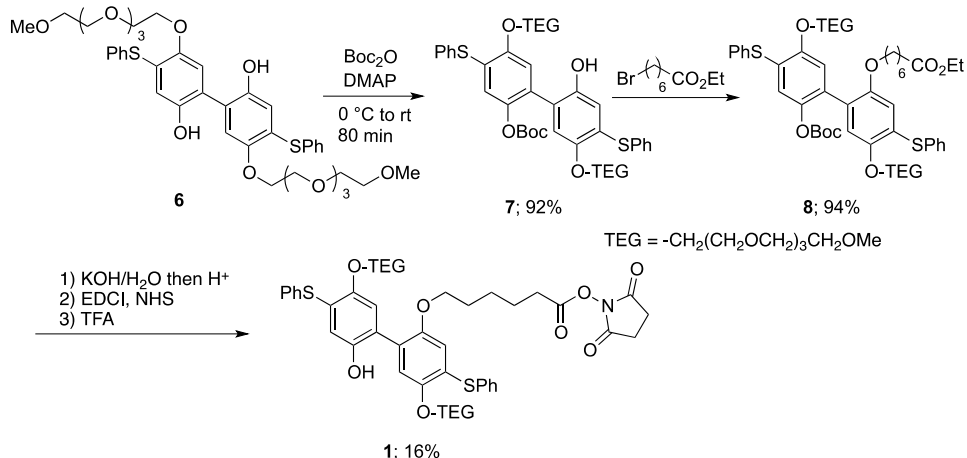
Scheme 3. Synthesis of Compound 2

TEG = -CH₂(CH₂OCH₂)₃CH₂OMe
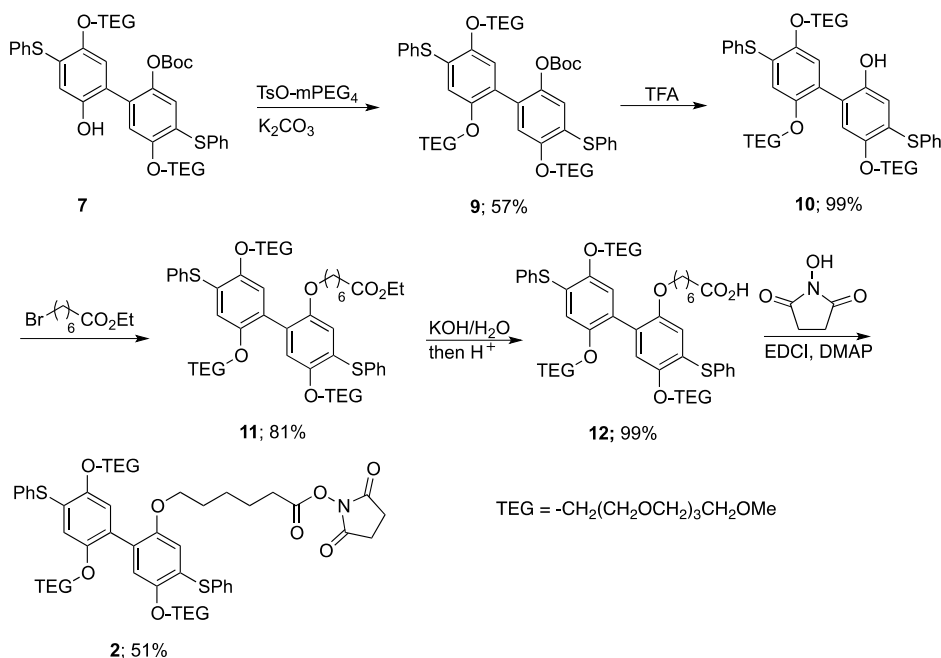
presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and catalytic amounts of *N,N*-dimethylaminopyridine (DMAP). Deprotection of the Boc unit by treatment with TFA gave the desired compound **1** in 16% yield over three steps (Scheme 2).

Preparation of compound **2** was carried out through the synthetic sequence shown in Scheme 3. The synthesis started from mono-Boc-protected **7**, to which the third tetraethylene glycol unit was introduced to give **9** in 57% yield. The Boc group in **9** was quantitatively removed by treatment with TFA, and the OH-containing compound **10** was obtained. Installation of the tether unit was achieved similarly as described for **8**, and compound **11** was obtained in 81% yield. Alkaline hydrolysis of the ethyl ester unit followed by esterification with NHS gave the desired compound **2** in 51% yield over two steps.

The photophysical properties of compounds **1** and **2** were examined. The UV absorption and fluorescence spectra in MeOH of compound **2** are shown in Figure 2. Compounds **1** and **2** produced similar UV absorption spectra in MeOH, with
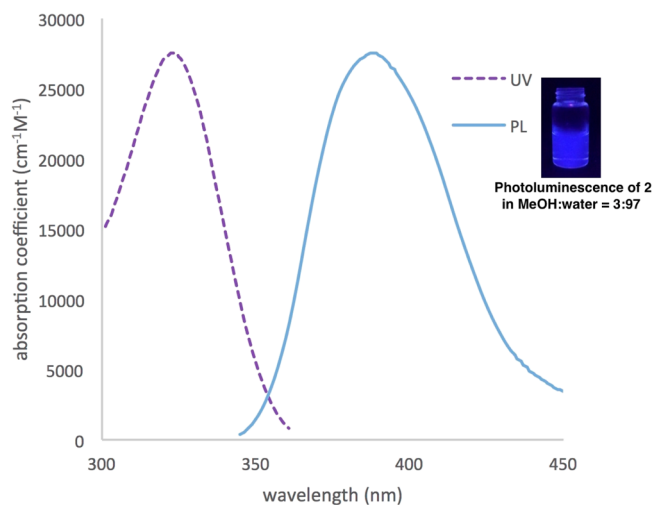

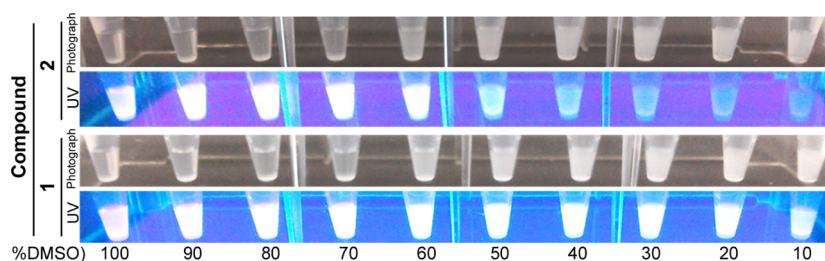**Figure 2.** UV and PL spectra of compound **2**.

Figure 3. Solubilities of compounds **1** and **2** at 10^{-3} M in water−DMSO solvents.
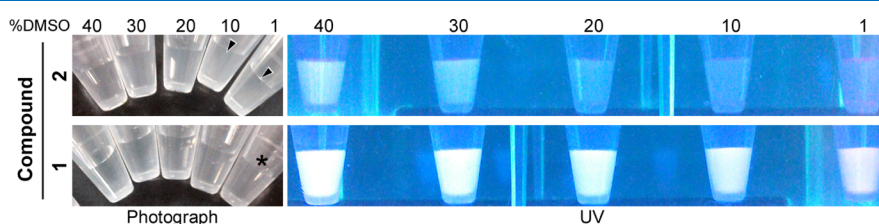

Figure 4. Solubilities of compounds **1** and **2** at a concentration of 10^{-4} M in DMSO−water mixtures.
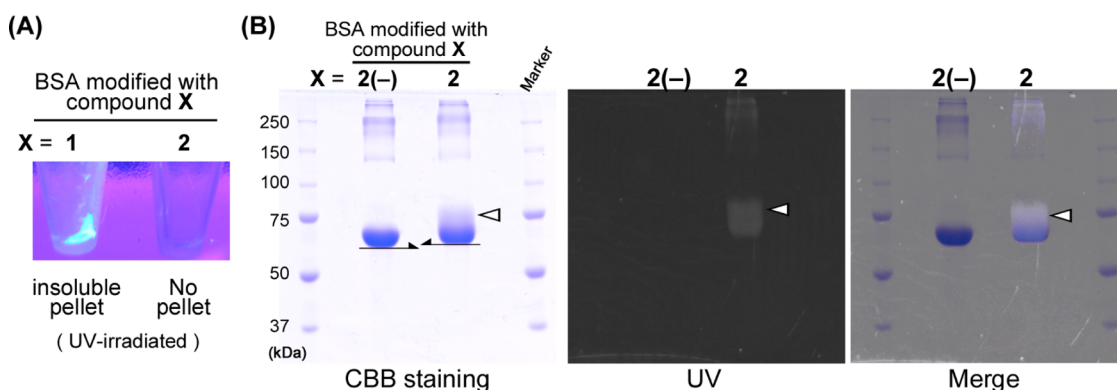

Figure 5. Fluorescence of BSA modified with the compounds. (A) Fluorescence emitted by an insoluble pellet generated by the chemical modification of BSA. (B) Fluorescence and CBB staining images of the modified BSA separated by SDS-PAGE.

the maximum absorbance (λ_{max}) at 324 nm for compound **1** and at 322 nm for compound **2**. Both compounds showed blue fluorescence on irradiation with UV light at each λ_{max}. Emission peaks were observed at 391 nm for compound **1** and at 388 nm for compound **2**. The quantum yields of the fluorescence were determined to be 0.158 and 0.168 for **1** and **2**, respectively. These photophysical properties are similar to those obtained for the starting compound **3**, demonstrating that the introduction of the tether and tetraethylene glycol units on the four phenolic OH groups did not affect the photophysical properties significantly.

To our delight, compound **2** was soluble in water containing small amounts of MeOH (97:3 v/v). UV and photo-luminescence (PL) spectra were measured in 10^{-4} and 10^{-5} M solutions of compound **2**, respectively. At these concentrations, homogeneous solutions were obtained in this mixed solvent system. The corresponding absorption maximum was observed at 318 nm, which was similar to that obtained in MeOH, whereas the emission peak appeared slightly red-shifted at 404 nm. The quantum yield of compound **2** in water/MeOH (97/3) was determined to be 0.205, slightly higher than that obtained in MeOH.

To investigate the potential applications of compounds **1** and **2** in bioimaging, we examined their reaction with BSA protein in water. To attach the protein, aqueous solutions of

these compounds at a concentration of at least 10^{-4} M should be prepared to ensure high reaction efficiency. We examined the solubility and fluorescence of compounds **1** and **2** in dimethyl sulfoxide (DMSO)/water solution in a concentration range from 10^{-3} M to 10^{-4} M. For the 10^{-3} M concentration, a homogeneous solution was obtained when the DMSO content was higher than 80%, whereas formation of a heterogeneous suspension was clearly observed for DMSO contents below 70% (Figure 3).[17]

Obvious turbidity was observed in samples prepared with 10% or less DMSO at a concentration of 10^{-4} M (Figure 4, indicated by arrowheads). The heterogeneous mixtures showed less fluorescence activity than the homogeneous ones (Figures 3 and 4), which should be due to the aggregation effect of the dye. It was found that compound **2** exhibited better solubility than compound **1**. Thus, solutions of compound **2** prepared in a mixed solvent system at a concentration of 10^{-4} M were homogeneous even for the DMSO content as low as 10% (Figure 4). A slight turbidity was observed with a DMSO content of 1% (Figure 4, indicated by an asterisk). In solutions with a low DMSO content, compound **2** showed a stronger fluorescence than compound **1** (Figures 3 and 4), which could be attributed to the higher solubility of the former.

To verify the utility of the compounds as amino group-reactive fluorescent probes in biochemical and biophysical applications, we attached the compounds to BSA and examined the fluorescence activities of the modified protein. As a preliminary test, the reagents including compound **1** (or **2**), BSA, and DMSO were mixed at concentrations of 10^{-4} M, 2×10^{-5} M, and 20%, respectively. This DMSO content ensured complete dissolution of the reactants. However, in the reaction with compound **1**, modified BSA became insoluble as the reaction progressed. In contrast, such insolubilization was not observed with compound **2**. Figure 5A shows the fluorescence emitted by an insoluble pellet of the modified BSA, which was obtained by centrifugation after the removal of unreacted compounds. This result suggests that the hydrophobicity of BSA increased by the reaction with compound **1**, with a concomitant reduction of the solubility of the modified BSA in aqueous solution. In addition to this insolubilizing effect, compound **1** has the disadvantage of requiring a high concentration of DMSO for complete dissolution, which tends to promote unfolding and denaturation of proteins.[18] Taken together, these results show that compound **1** did not have sufficient water solubility to enable its utilization in biochemical experiments. Next, we modified BSA with compound **2** at a sufficiently low DMSO concentration to prevent protein denaturation. The modified BSA was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the in-gel fluorescence of BSA was then detected using a UV Gel Imager. Total proteins were stained with the Coomassie brilliant blue (CBB) dye. The bands of BSA were detected at appropriate locations corresponding to the molecular weight (69 kDa) of BSA (Figure 5B, "CBB"). The band of BSA treated with compound **2** slightly shifted upward, as compared with that of a negative control consisting of BSA modified with compound **2** without the amine-reactive succinimidyl ester (Figure 5B, arrows). BSA modified with compound **2** showed a clear fluorescence, whereas no fluorescence was observed in the negative control (Figure 5B, "UV"). The CBB-stained band of **2**-modified BSA formed a smear with a weaker staining intensity from the bottom to the top of the band. In contrast, such smear was not observed in the negative control. Meanwhile, the UV image showed a strong fluorescence intensity without any fading in the upper region of the smeared band of **2**-modified BSA (Figure 5B, indicated by arrowheads). This is probably due to multiple binding of compound **2** to a single molecule of BSA. These results demonstrate that compound **2** chemically binds to BSA and emits fluorescence. Furthermore, compound **2** exhibited a high stability with no loss of fluorescence, even after standing in DMSO solution for at least 1 month at room temperature (data not shown).

CONCLUSIONS

The solubility of 2-phenylsulfanylhydroquinone dimer in water was successfully enhanced by attaching tetraethylene glycol units. To achieve sufficient water solubility, three tetraethylene glycol units should be introduced as side chains. This modification did not alter significantly the photophysical properties of the compound, and good blue fluorescence and quantum yields ranging between 0.122 and 0.226 were observed in MeOH solution. Trisubstituted derivative **2** showed good solubility in the MeOH/water (3:97) solution, which emitted blue light at 404 nm upon irradiation with 318 nm UV light.

Moreover, it was demonstrated that the introduction of a NHS-terminated C6-side chain was suitable for the attachment of peptide units such as BSA. The resulting BSA−dye **2** complex showed nice blue fluorescence, indicating that the 2-phenylsulfanylhydroquinone dimer is potentially useful as a new bioimaging material. The introduction of the protein to compound **2** progressed under mild conditions, thereby preventing protein denaturation. Modification of compound **1** with BSA caused insolubilization, which was not observed in the case of compound **2**. These results suggest that the number of modifying groups can control the water solubility of this fluorescent molecule and modulate the physicochemical properties imparted to the protein.

Our current results constitute a primary example of tuning of the properties of 2-phenylsulfanylhydroquinone dyes by chemical modification and opened a new avenue for further modification of the dye. Investigation on the changes of its photophysical properties is currently underway in our laboratory.

EXPERIMENTAL SECTION

General. All ^{1}H and ^{13}C NMR spectra were recorded on a JEOL LAMDA-500 or JNM-ECA 500 Delta2 (500 MHz for ^{1}H and 126 MHz for ^{13}C) spectrometer. High-resolution mass spectra were measured by a JEOL JMS T-100LP LC-ESI mass spectrometer. UV−vis spectra were measured by a SHIMAD-ZU UV-1650PC spectrometer. Fluorescence spectra were measured by a JASCO FP-6200 fluorescence spectrometer. Absolute quantum yields were measured by a Hamamatsu Photonics C9920-02G absolute quantum yield spectrometer. All reagents used in this work were purchased from Aldrich or Tokyo Kasei Co., Ltd. and used without further purification. All the reactions in this paper were performed under nitrogen atmosphere unless otherwise mentioned. Compound **4** was prepared by the procedure previously reported.[15]

Preparation of 2-Phenylthiohydroquinone Dimer Dimethyl Ketal Bis(tetraethylene glycol monomethylether) 5. Compound **4** (527 mg, 1.008 mmol) was added to a mixture of K_2CO_3 (418 mg, 3.023 mmol), KI (335 mg, 2.016 mmol), and 18-crown-6 (33 mg, 0.504 mmol) in tetrahydrofuran (THF) (3 mL). To the reaction mixture, tetraethylene glycol monomethylether tosylate (1.096 g, 3.023 mmol) was added. The reaction mixture was stirred at refluxing temperature for 72 h. After cooling down, water (10 mL) was added, and the resulting mixture was extracted with EtOAc (10 mL × 10). The organic phase was combined, washed with brine (30 mL), and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo and the residue was subjected to flash chromatography (silica gel/EtOAc) to give compound **5** in 85% yield (732 mg, 0.856 mmol). Pale amber oil. ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.43−7.24 (m, 10H), 6.96 (s, 2H), 6.76 (s, 2H), 4.21 (t, *J* = 9.9 Hz, 4H), 3.83 (t, *J* = 5.0 Hz, 4H), 3.76−3.67 (m, 4H), 3.68−3.60 (m, 16H), 3.57−3.48 (m, 4H), 3.36 (s, 6H), 1.45 (s, 6H); ^{13}C NMR (126 MHz, CDCl$_3$): δ 153.8, 145.7, 134.1, 132.1, 132.0, 129.4, 127.6, 126.1, 125.4, 115.7, 111.6, 72.0, 71.1, 70.8, 70.7, 70.7, 70.6, 69.6, 69.5, 59.1, 24.7; HRMS (ESI-TOF): calcd for $C_{45}H_{58}NaO_{12}S_2$, 877.3267 [M + Na$^+$]; found, 877.3238.

Preparation of 2-Phenylthiohydroquinone Dimer Bis-(tetraethylene glycol monomethylether) 6. Compound **5** (1.314 g, 1.537 mmol) was dissolved in THF (2 mL)−concentrated HCl (1.5 mL). The reaction mixture was stirred at room temperature for 18 h. Water (10 mL) was added, and

the resulting mixture was extracted with EtOAc (10 mL × 10). The organic phase was combined, washed with brine (30 mL), and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo and the residue was subjected to flash chromatography (silica gel/EtOAc) to give compound **6** in 98% yield (1.217 g, 1.493 mmol). Pale amber oil. ^1H NMR (500 MHz, $CDCl_3$): δ 7.48−7.42 (m, 4H), 7.37−7.27 (m, 6H), 6.89−6.84 (m, 2H), 6.83 (s, 2H), 6.54 (s, 2H), 4.14 (t, J = 4.9 Hz, 4H), 3.76 (t, J = 4.8 Hz, 4H), 3.68−3.62 (m, 4H), 3.59−3.49 (m, 16H), 3.50−3.42 (m, 4H), 3.28 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 150.3, 147.7, 133.4, 133.1, 129.4, 127.8, 127.4, 124.1, 118.5, 115.7, 71.9, 71.0, 70.7, 70.6, 70.5, 70.4, 70.0, 69.7, 59.0; HRMS (ESI-TOF): calcd for $C_{42}H_{54}NaO_{12}S_2$ 837.2954 [M + Na$^+$]; found, 837.2967.

Preparation of Mono-Boc Protected 2-Phenylthiohydroquinone Dimer Bis(tetraethylene glycol monomethylether) 7. Compound **6** (1.477 g, 1.812 mmol) was dissolved in CH_2Cl_2 (6 mL), and Boc_2O (397 mg, 1.812 mmol) and DMAP (45.0 mg, 0.3624 mmol) were added to the solution at 0 °C. The reaction mixture was stirred at the same temperature for 80 min. The solvent was removed in vacuo, and the residue was subjected to flash chromatography (silica gel/EtOAc) to give compound **7** in 92% yield (1.522 g, 1.663 mmol). Pale yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ 7.48 (d, J = 9.6 Hz, 1H), 7.44−7.41 (m, 2H), 7.39−7.30 (m, 7H), 6.85 (s, 1H), 6.71 (s, 1H), 6.67 (s, 1H), 6.56 (s, 1H), 5.18 (s, 1H), 4.17 (d, J = 9.7 Hz, 2H), 4.09 (t, J = 9.9 Hz, 2H), 3.82 (d, J = 9.7 Hz, 2H), 3.79 (t, J = 9.9 Hz, 2H), 3.75−3.68 (m, 4H), 3.67−3.59 (m, 16H), 3.56−3.47 (m, 4H), 3.36 (s, 3H), 3.34 (s, 3H), 1.27 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 153.6, 151.8, 149.8, 147.7, 142.8, 133.6, 133.2, 133.2, 132.8, 132.3, 129.6, 129.4, 128.4, 128.3, 128.0, 128.0, 122.8, 122.3, 118.2, 114.9, 114.6, 84.0, 72.0, 72.0, 71.2, 71.1, 70.75, 70.74, 70.72, 70.71, 70.67, 70.6, 70.6, 70.5, 69.7, 69.7, 69.6, 69.4, 59.1, 59.1, 27.4; HRMS (ESI-TOF): calcd for $C_{47}H_{62}NaO_{14}S_2$ 937.3479 [M + Na$^+$]; found, 937.3441.

Preparation of Compound 8. Compound **7** (467 mg, 0.511 mmol) was dissolved in THF (2 mL), and tBuOK (68.7 mg, 0.6126 mmol) and ethyl 6-bromocaproate (123 mg, 0.5616 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 45 h. 1 M HCl aq (10 mL) was added to the reaction mixture, and the resulting solution was extracted with EtOAc (10 mL × 10). The organic phase was combined, washed with brine (30 mL), and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo, and the residue was subjected to flash chromatography (silica gel/EtOAc) to give O-caproate derivative in 94% yield (506 mg, 0.478 mmol). Pale yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ 7.43 (d, J = 6.9 Hz, 2H), 7.39−7.26 (m, 8H), 6.87 (s, 1H), 6.81 (s, 1H), 6.76 (s, 1H), 6.65 (s, 1H), 4.14−4.06 (m, 6H), 3.80 (t, J = 5.5 Hz, 2H), 3.74 (t, J = 4.9 Hz, 2H), 3.70 (dd, J = 5.1, 4.4 Hz, 2H), 3.68−3.58 (m, 22H), 3.54−3.51 (m, 4H), 3.36 (s, 3H), 3.36 (s, 3H), 2.19 (d, J = 7.7 Hz, 2H), 1.56−1.48 (m, 4H), 1.26 (s, 9H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.7, 153.2, 151.3, 150.7 (2C), 142.6, 134.5, 133.3, 132.8 (2C), 131.8 (2C), 129.9, 129.4 (2C), 129.3 (2C), 127.8, 127.4, 126.0, 126.0, 125.4, 123.3, 117.1, 115.9, 114.7, 82.9, 72.0 (2C), 71.1, 71.0, 70.7, 70.7, 70.7 (2C), 70.7 (2C), 70.6 (2C), 69.7, 69.6, 69.5, 69.5, 69.3, 60.3, 59.1, 34.3, 28.8, 27.5 (3C), 25.4, 24.6, 14.4; HRMS (ESI-TOF): calcd for $C_{55}H_{76}NaO_{16}S_2$, 1079.4472 [M + Na$^+$]; found, 1079.4482.

Preparation of Compound 1. Compound **8** (231.1 mg, 0.214 mmol) was dissolved in THF. KOH aq (24.9 mg, 0.437

mmol in 2 mL of water) was added to the solution, and the reaction mixture was heated to refluxing temperature for 5 h. 1 M HCl aq (10 mL) was added, and MeOH was removed in vacuo. The resulting aqueous mixture was extracted with EtOAc (10 mL × 10). The organic phase was combined, washed with brine (30 mL), and dried over Na_2SO_4. After filtration, the removal of the solvent gave crude carboxylic acid derivative in 91% yield (pale yellow oil, 203.6 mg, 0.194 mmol), which was used for the next step without further purification.

Carboxylic acid (191 mg, 0.186 mmol) was dissolved in THF (2 mL). EDCI·HCl (55.0 mg, 0.278 mmol) and NHS (34.0 mg, 0.278 mmol) were added to the solution. The reaction mixture was stirred at 50 °C for 47 h. NH_4Cl aq (10 mL) was added to the mixture, and the mixture was extracted with EtOAc (10 mL × 5). The organic phase was combined, washed with brine (30 mL), and dried over Na_2SO_4. After filtration, the removal of the solvent gave crude NHS amide derivative in 28% yield (pale yellow oil, 58.1 mg, 0.0516 mmol), which was used for the final step of Boc removal without further purification.

NHS amide (58.1 mg, 0.0525 mmol) was dissolved in CH_2Cl_2 (3 mL), and TFA (50 mg) and anisole (109 mg) were added to the solution. The reaction mixture was stirred for 21 h at room temperature. The reaction mixture was concentrated in vacuo, and the residue was subjected to flash chromatography (silica gel/EtOAc containing 2% of MeOH) to give compound **1** in 57% yield (30.6 mg, 0.0298 mmol). Pale yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ 7.45 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H), 7.37−7.27 (m, 6H), 6.85 (s, 1H), 6.77 (s, 1H), 6.66 (s, 1H), 6.59 (s, 1H), 6.48 (s, 1H), 4.14 (t, J = 4.8 Hz, 4H), 3.83−3.59 (m, 26H), 3.54−3.50 (m, 4H), 3.34 (s, 6H), 2.83−2.80 (m, 4H), 2.49 (d, J = 7.4 Hz, 2H), 1.66−1.55 (m, 4H), 1.36−1.29 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.3 (2C), 168.5, 151.8, 150.1, 149.1, 148.4, 133.8, 133.2 (3C), 132.4 (2C), 129.5 (2C), 129.5 (2C), 128.0, 127.8, 127.7, 127.2, 126.6, 124.4, 119.0, 117.2, 116.2, 115.1, 72.0 (2C), 71.1 (2C), 70.7, 70.7 (2C), 70.7 (2C), 70.6 (2C), 70.6 (2C), 69.7 (2C), 69.7, 69.6, 59.1 (2C), 30.8, 28.6, 25.7 (2C), 25.0, 24.2; HRMS (ESI-TOF): calcd for $C_{52}H_{67}N_1NaO_{16}S_2$, 1048.3798 [M + Na$^+$]; found, 1048.3798.

Preparation of Mono-Boc-Protected 2-Phenylthiohydroquinone Dimer Tris(tetraethylene glycol monomethylether) 9. Compound **7** (1.214 g, 1.326 mmol) was dissolved in THF (6 mL), and K_2CO_3 (642 mg, 2.652 mmol), KI (771 mg, 2.652 mmol), and 18-crown-6 (302 mg, 0.6631 mmol) were added to the solution. To the mixture, tetraethylene glycol monomethylether tosylate (1.262 g, 3.48 mmol) was added. The reaction mixture was stirred at refluxing temperature for 23 h. After cooling down, water (10 mL) was added, and the resulting mixture was extracted with EtOAc (10 mL × 10). The organic phase was combined, washed with brine (30 mL), and dried over Na_2SO_4. After filtration, the solvent was removed in vacuo, and the residue was subjected to flash chromatography (silica gel/EtOAc containing 2% of MeOH) to give compound **9** in 57% yield (35 mg, 0.7554 mmol). Pale yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ 7.43 (d, J = 6.9 Hz, 2H), 7.38−7.26 (m, 8H), 6.92 (s, 1H), 6.81 (s, 1H), 6.76 (s, 1H), 6.72 (s, 1H), 4.13 (t, J = 4.9 Hz, 2H), 4.08 (d, J = 4.9 Hz, 2H), 3.84−3.46 (m, 44H), 3.36 (s, 6H), 3.35 (s, 3H), 1.26 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 153.2, 151.2, 151.0, 150.5, 142.5, 134.3, 133.2, 132.8 (2C), 131.9 (2C), 129.6, 129.4 (2C), 129.3 (2C), 127.9, 127.5, 126.1, 126.0, 125.6,

123.1, 117.8, 115.6, 114.8, 82.9, 72.0 (3C), 71.1, 71.0, 70.7, 70.7, 70.6 (2C), 70.6 (2C), 70.6, 70.5 (4C), 69.7, 69.6, 69.5 (2C), 69.4, 69.3, 59.1 (3C), 27.4 (3C); HRMS (ESI-TOF): calcd for $C_{56}H_{80}NaO_{18}S_2$, 1127.4684 [M + Na$^+$]; found, 1127.4680.

Preparation of 2-Phenylthiohydroquinone Dimer Tris-(tetraethylene glycol monomethylether) 10. Compound **9** (835 mg, 0.755 mmol) was dissolved in CH_2Cl_2 (3 mL), and TFA (430.5 mg, 3.78 mmol) and anisole (326.6 mg, 3.02 mmol) were added to the solution. The reaction mixture was stirred for 72 h at room temperature. The reaction mixture was concentrated in vacuo, and the residue was subjected to flash chromatography (silica gel/EtOAc containing 2% of MeOH) to give compound **10** in 99% yield (753 mg, 0.749 mmol). Pale yellow oil. ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.43−7.26 (m, 10H), 6.86 (s, 1H), 6.76 (s, 1H), 6.70−6.66 (m, 2H), 4.16−4.11 (m, 4H), 3.94−3.90 (m, 2H), 3.84−3.78 (m, 3H), 3.74−3.49 (m, 40H), 3.37 (s, 6H), 3.33 (s, 3H); ^{13}C NMR (126 MHz, CDCl$_3$): δ 151.7, 150.6, 149.2, 148.5, 134.1, 133.9, 132.4 (2C), 131.9 (2C), 129.4 (4C), 129.3 (4C), 127.6 (2C), 127.4, 125.8, 120.7, 116.2 (2C), 115.1, 71.9, 71.9 (2C), 71.0 (2C), 70.7, 70.6 (2C), 70.6 (2C), 70.5, 70.5 (2C), 70.5, 70.4 (2C), 69.8, 69.8 (2C), 69.7, 69.2, 69.1, 59.2, 59.2, 59.1; HRMS (ESI-TOF): calcd for $C_{51}H_{72}NaO_{16}S_2$, 1027.4159 [M + Na$^+$]; found, 1027.4147.

2-Phenylthiohydroquinone Dimer Tris(tetraethylene glycol monomethylether) O-6-Capronic Acid Ethyl Ester 11. Compound **10** (813 mg, 0.8086 mmol) was dissolved in THF (2 mL), and tBuOK (111 mg, 0.970 mmol) and ethyl 6-bromocaproate (200 mg, 0.8895 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 120 h. 1 M HCl aq (10 mL) was added to the reaction mixture, and the resulting solution was extracted with EtOAc (10 mL × 5). The organic phase was combined, washed with brine (30 mL), and dried over Na$_2$SO$_4$. After filtration, the solvent was removed in vacuo, and the residue was subjected to flash chromatography (silica gel/EtOAc) to give compound **11** in 81% yield (750 mg, 0.746 mmol). Pale yellow oil. ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.39−7.24 (m, 10H), 6.87 (s, 1H), 6.85 (s, 1H), 6.71 (s, 1H), 6.66 (s, 1H), 4.09 (q, J = 7.1 Hz, 2H), 4.07 (t, J = 5.3 Hz, 4H), 3.81 (t, J = 5.6 Hz, 2H), 3.77−3.72 (m, 4H), 3.69−3.43 (m, 40H), 3.36 (s, 6H), 3.35 (s, 3H), 2.19 (t, J = 7.5 Hz, 2H), 1.55−1.47 (m, 4H), 1.29−1.17 (m, 2H), 1.23 (t, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, CDCl$_3$): δ 173.6, 150.9, 150.8, 150.8, 150.6, 134.9, 134.8, 131.47 (2C), 131.44 (2C), 129.25 (2C), 129.22 (2C), 127.4, 127.22, 127.18 (2C), 124.4, 124.3, 117.0, 116.5 (2C), 116.4, 72.00 (2C), 71.99, 71.04, 71.02, 70.75, 70.72 (2C), 70.68 (2C), 70.63 (2C), 70.62, 70.61, 70.59, 70.58 (2C), 70.57, 69.69, 69.64, 69.62, 69.61, 69.55, 69.3, 69.1, 60.3, 59.1 (3C), 34.2, 29.0, 25.6, 24.7, 14.4; HRMS (ESI-TOF): calcd for $C_{59}H_{86}NaO_{18}S_2$, 1169.5153 [M + Na$^+$]; found, 1169.5182.

Hydrolysis of Compound 11. Compound **11** (750 mg, 0.6535 mmol) was dissolved in THF. KOH aq (328 mg, 5.847 mmol in 2 mL of water) was added to the solution, and the reaction mixture was heated to refluxing temperature for 23 h. 1 M HCl aq (10 mL) was added, and MeOH was removed in vacuo. The resulting aqueous mixture was extracted with EtOAc (10 mL × 10). The organic phase was combined, washed with brine (30 mL), and dried over Na$_2$SO$_4$. After filtration, the removal of the solvent gave crude carboxylic acid derivative **12** in 91% yield (744 mg, 0.6647 mmol). Pale yellow oil. ^{1}H NMR (500 MHz, CDCl$_3$): δ 10.09−9.09 (m, 1H), 7.38

(d, J = 8.3 Hz, 4H), 7.32 (td, J = 7.5, 2.2 Hz, 4H), 7.27−7.25 (m, 2H), 6.88 (s, 1H), 6.85 (s, 1H), 6.73 (s, 1H), 6.67 (s, 1H), 4.07 (t, J = 5.0 Hz, 4H), 3.82−3.46 (m, 46H), 3.36 (s, 3H), 3.36 (s, 6H), 2.21 (t, J = 7.0 Hz, 2H), 1.56−1.48 (m, 4H), 1.30−1.24 (m, 2H); ^{13}C NMR (126 MHz, CDCl$_3$): δ 166.1, 150.84, 150.79, 150.72, 150.5, 134.9, 134.8, 131.5 (2C), 131.4 (2C), 129.3 (2C), 129.2 (2C), 127.5, 127.2 (2C), 127.1, 127.0, 124.3, 117.3, 116.6, 116.4 (2C), 72.0, 71.9 (2C), 71.0 (2C), 70.72, 70.69, 70.68, 70.65, 70.63 (2C), 70.62, 70.60, 70.58, 70.57, 70.56, 70.52, 70.50, 69.66, 69.64 (2C), 69.60, 69.34, 69.27, 69.0, 59.1 (3C), 33.9, 29.0, 25.8, 24.7; HRMS (ESI-TOF): calcd for $C_{57}H_{82}NaO_{18}S_2$, 1141.4840 [M + Na$^+$]; found, 1141.4846.

Preparation of Compound 2. The carboxylic acid derivative **12** (744 mg, 0.6645 mmol) was dissolved in THF (2 mL). EDCI·HCl (195 mg, 0.9967 mmol) and NHS (119 mg, 0.9967 mmol) were added to the solution. The reaction mixture was stirred at 50 °C for 21 h. NH$_4$Cl aq (10 mL) was added to the mixture, and the mixture was extracted with EtOAc (10 mL × 5). The organic phase was combined, washed with brine (30 mL), and dried over Na$_2$SO$_4$. After filtration, the removal of the solvent gave crude compound **2**, which was purified in a GPC apparatus to give compound **2** in 51% yield (415 mg, 0.3414 mmol). Pale yellow oil. ^{1}H NMR (500 MHz, CDCl$_3$): δ 7.37 (d, J = 8.1 Hz, 4H), 7.32 (td, J = 7.5, 3.5 Hz, 4H), 7.29−7.23 (m, 2H), 6.86 (s, 1H), 6.84 (s, 1H), 6.71 (s, 1H), 6.66 (s, 1H), 4.07 (t, J = 4.9 Hz, 4H), 3.82−3.79 (m, 2H), 3.76−3.72 (m, 4H), 3.69−3.43 (m, 40H), 3.36 (s, 3H), 3.35 (s, 3H), 3.35 (s, 3H), 2.82 (d, J = 4.7 Hz, 4H), 2.49 (t, J = 7.4 Hz, 2H), 1.64−1.58 (m, 2H), 1.57−1.50 (m, 2H), 1.33−1.26 (m, 2H); ^{13}C NMR (126 MHz, CDCl$_3$: δ 169.3 (2C), 168.5, 150.84, 150.81, 150.76, 150.6, 134.8, 134.7, 131.54 (2C), 131.51 (2C), 129.27 (2C), 129.25 (2C), 127.4, 127.3, 127.24, 127.21, 124.5, 124.4, 117.0, 116.50, 116.47, 116.3, 72.0 (3C), 71.0 (2C), 70.74, 70.73 (2C), 70.68 (2C), 70.64, 70.62 (3C), 70.59 (4C), 69.7, 69.64, 69.62, 69.60, 69.5, 69.3, 69.0, 59.1 (3C), 30.9, 28.8, 25.7 (2C), 25.3, 24.3; HRMS (ESI-TOF): calcd for $C_{61}H_{85}NNaO_{20}S_2$, 1238.5004 [M + Na$^+$]; found, 1238.4999.

Evaluation of Solubility. The solubilities (the generation of insoluble precipitates) of compounds **1** and **2** in water−DMSO were determined by observing the pellet formation after centrifugation (at 3000*g* for 3 min at room temperature).

Observation of Fluorescence Emitted from Compounds 1 and 2 in DMSO Solution. Compounds **1** and **2** in water−DMSO solutions were excited with ultraviolet light (312 nm) using a UV irradiator DT-20MP (ATTO, Tokyo, Japan), and the emitted blue fluorescence was photographed.

Conjugation of the Compounds to BSA. Compounds **1** or **2** (2 μL, 5 mM in DMSO) and BSA (6 μL, 20 mg/mL in PBS) were incubated in 0.1 mL of 0.1 M NaHCO$_3$ aq for 6 h at 4 °C. The final concentration of DMSO was 2%. The molar ratio of the compound and BSA was 10:2. The reaction was stopped by adding 1 μmol of ethanolamine (Sigma-Aldrich, St. Louis, US-MO). The unreacted compound was removed by ultrafiltration using Amicon Ultra centrifugal filter devices with 10 kDa MWCO (Merck Millipore, Darmstadt, Germany). Compound **2**(−), pretreated with ethanolamine to eliminate the reactivity of succinimidyl ester, was reacted with BSA as a negative control.

Observation of Fluorescence Emitted from Compound 2-Modifed BSA. A 2 μg of the modified BSA was subjected to SDS-PAGE (9%). The fluorescence of the

modified BSA in the gel was detected by a UV-transilluminator (302 nm) using a FluorChem FC2 Gel Imager (Alpha Innotech, Hessisch Oldendorf, Germany). Thus, the total proteins were stained with 0.1% CBB-R250.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c00703.

NMR and UV/PL spectra of the compounds (PDF)

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The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

DEDICATION

We dedicate this paper to Professor Ilhyong Ryu on the occasion of his 70th birthday.

REFERENCES

(1) (a) Grimm, J. B.; Heckman, L. M.; Lavis, L. D. The Chemistry of Small-Molecule Fluorogenic Probes. *Prog. Mol. Biol. Transl. Sci.* **2013**, *113*, 1. (b) Kim, E.; Park, S. B. Chemistry as a Prism: A Review of Light−Emitting Materials Having Tunable Emission Wavelengths. *Chem.—Asian J.* **2009**, *4*, 1646−1658. (c) Dsouza, R. N.; Pischel, U.; Nau, W. M. Fluorescent Dyes and Their Supramolecular Host/Guest Complexes with Macrocycles in Aqueous Solution. *Chem. Rev.* **2011**, *111*, 7941−7980. (d) Martinez, V.; Henary, M. Nile Red and Nile Blue: Applications and Syntheses of Structural Analogues. *Chem.—Eur. J.* **2016**, *22*, 13764−13782. (e) Guo, Z.; Park, S.; Yoon, J.; Shin, I. Recent progress in the development of near-infrared fluorescent probes for bioimaging applications. *Chem. Soc. Rev.* **2014**, *43*, 16−29.

(2) Bi, A.; Yang, S.; Liu, M.; Wang, X.; Liao, W.; Zeng, W. Fluorescent probes and materials for detecting formaldehyde: from laboratory to indoor for environmental and health monitoring. *RSC Adv.* **2017**, *7*, 36421−36432.

(3) Royo, S.; Martínez-Máñez, R.; Sancenón, F.; Costero, A. M.; Parra, M.; Gil, S. Chromogenic and fluorogenic reagents for chemical warfare nerve agents' detection. *Chem. Commun.* **2007**, 4839−4847.

(4) Klymchenko, A. S. Solvatochromic and Fluorogenic Dyes as Environment-Sensitive Probes: Design and Biological Applications. *Acc. Chem. Res.* **2017**, *50*, 366−375.

(5) Du, J.; Hu, M.; Fan, J.; Peng, X. Fluorescent chemodosimeters using "mild" chemical events for the detection of small anions and cations in biological and environmental media. *Chem. Soc. Rev.* **2012**, *41*, 4511−4535.

(6) Kolusheva, S.; Molt, O.; Herm, M.; Schrader, T.; Jelinek, R. Selective Detection of Catecholamines by Synthetic Receptors Embedded in Chromatic Polydiacetylene Vesicles. *J. Am. Chem. Soc.* **2005**, *127*, 10000−10001.

(7) Kenmoku, S.; Urano, Y.; Kojima, H.; Nagano, T. Development of a Highly Specific Rhodamine-Based Fluorescence Probe for Hypochlorous Acid and Its Application to Real-Time Imaging of Phagocytosis. *J. Am. Chem. Soc.* **2007**, *129*, 7313−7318.

(8) Asanuma, D.; Sakabe, M.; Kamiya, M.; Yamamoto, K.; Hiratake, J.; Ogawa, M.; Kosaka, N.; Choyke, P. L.; Nagano, T.; Kobayashi, H.; Urano, Y. Sensitive β-galactosidase-targeting fluorescenceprobe for visualizing small peritoneal metastatictumours in vivo. *Nat. Commun.* **2015**, *6*, 6463. Parasar, B.; Chang, P. V. Finding the Sweet Spot for Breast Cancer Detection. *ACS Cent. Sci.* **2020**, *6*, 2123−2125.

(9) Some examples using coumarin; see, (a) Begoyan, V. V.; Weseliński, Ł. J.; Xia, S.; Fedie, J.; Kannan, S.; Ferrier, A.; Rao, S.; Tanasova, M. Multicolor GLUT5-permeable fluorescent probes for fructose transport analysis. *Chem. Commun.* **2018**, *54*, 3855−3858. Long, L.; Huang, M.; Wang, N.; Wu, Y.; Wang, K.; Gong, A.; Zhang, Z.; Sessler, J. L. A Mitochondria-Specific Fluorescent Probe for Visualizing Endogenous Hydrogen Cyanide Fluctuations in Neurons. *J. Am. Chem. Soc.* **2018**, *140*, 1870−1875. (c) Ong, J. X.; Pang, V. Y. T.; Tng, L. M.; Ang, W. H. Pre−Assembled Coumarin−Rhodamine Scaffold for Ratiometric Sensing of Nitric Oxide and Hypochlorite. *Chem.—Eur. J.* **2018**, *24*, 1870−1876. (d) Lee, J. H.; Kim, K. Y.; Jin, H.; Baek, Y. E.; Choi, Y.; Jung, S. H.; Lee, S. S.; Bae, J.; Jung, J. H. Self-Assembled Coumarin Nanoparticle in Aqueous Solution as Selective Mitochondrial-Targeting Drug Delivery System. *ACS Appl. Mater. Interfaces* **2018**, *10*, 3380−3391. (e) Abdul Manaf, S. A.; Hegde, G.; Mandal, U. K.; Wui, T. W.; Roy, P. Functionalized Carbon Nano-scale Drug Delivery Systems From Biowaste Sago Bark For Cancer Cell Imaging. *Curr. Drug Deliv.* **2017**, *14*, 1071−1077. (f) Yang, Y.; Wang, H.; Wei, Y.-L.; Zhou, J.; Zhang, J.-F.; Zhou, Y. A selective coumarin-based "turn-on" fluorescent sensor for the detection of cysteine and its applications for bioimaging. *Chin. Chem. Lett.* **2017**, *28*, 2023−2026.

(10) (a) Hsiang, J.-C.; Jablonski, A. E.; Dickson, R. M. Optically Modulated Fluorescence Bioimaging: Visualizing Obscured Fluorophores in High Background. *Acc. Chem. Res.* **2014**, *47*, 1545−1554. Wang, H.-Y.; Zhang, H.; Chen, S.; Liu, Y. Fluorescein-Inspired Near-Infrared Chemodosimeter for Luminescence Bioimaging. *Curr. Med. Chem.* **2019**, *26*, 4029−4041.

(11) (a) Jose, J.; Burgess, K. Benzophenoxazine-based fluorescent dyes for labelingbiomolecules. *Tetrahedron* **2006**, *62*, 11021−11037. (b) Qi, S.; Kim, S.; Nguyen, V.-N.; Kim, Y.; Niu, G.; Kim, G.; Kim, S.-J.; Park, S.; Yoon, J. Highly Efficient Aggregation-Induced Red-Emissive Organic Thermally Activated Delayed Fluorescence Materials with Prolonged Fluorescence Lifetime for Time-Resolved Luminescence Bioimaging. *ACS Appl. Mater. Interfaces* **2020**, *12*, 51293−51301. (c) Ravichandiran, P.; Boguszewska-Czubara, A.; Masłyk, M.; Bella, A. P.; Johnson, P. M.; Subramaniyan, S. A.; Shim, K. S.; Yoo, D. J. A phenoxazine-based fluorescent chemosensor for dual channel detection of Cd^{2+} and CN^-ions and its application to bio-imaging in live cells and zebrafish. *Dyes Pigments* **2020**, *172*, 107828. (d) Zhan, Y.-H.; Sun, R.; Zhu, W.-J.; Xu, Y.-J.; Ge, J.-F. An oxazine-based near-infrared fluorescent probe for selective incellular imaging of exogenous nitrite. *Sens. Actuators, B* **2017**, *240*, 1283−1290.

(12) (a) Gorka, A. P.; Nani, R. R.; Schnermann, M. J. Cyanine polyene reactivity: scope and biomedical applications. *Org. Biomol. Chem.* **2015**, *13*, 7584−7598. (b) Huang, S.; Liu, W.; Huang, J.; Wang, X.; Yang, C.; Bohra, H.; Liu, Q.; Wang, M. Theranostic Colloidal Nanoparticles of Pyrrolopyrrole Cyanine Derivatives for Simultaneous Near-Infrared Fluorescence Cancer Imaging and Photothermal Therapy. *ACS Appl. Bio Mater.* **2018**, *1*, 1109−1117. (c) Ruan, Z.; Yuan, P.; Jing, T.; Xing, T.; Yan, L. pH-Sensitive Polypeptide Conjugated with Carborane Clusters and Cyanine for NIR Bioimaging and Multi-Therapies. *Macromol. Res.* **2018**, *26*, 270−277. (d) Tan, X.; Constantin, T. P.; Sloane, K. L.; Waggoner, A. S.; Bruchez, M. P.; Armitage, B. A. Fluoromodules Consisting of a Promiscuous RNA Aptamer and Red or Blue Fluorogenic Cyanine Dyes: Selection, Characterization, and Bioimaging. *J. Am. Chem. Soc.* **2017**, *139*, 9001−9009. (e) Hu, F.; Jiang, L.; Cao, M.; Xu, Z.; Huang, J.; Wu, D.; Yang, W.; Liu, S. H.; Yin, J. Cyanine-based dithienylethenes: synthesis, characterization, photochromism and biological imaging in living cells. *RSC Adv.* **2015**, *5*, 5982−5987.

(13) (a) Bertrand, B.; Passador, K.; Goze, C.; Denat, F.; Bodio, E.; Salmain, M. Metal-based BODIPY derivatives as multimodal tools for life sciences. *Coord. Chem. Rev.* **2018**, *358*, 108−124. (b) Marfin, Y. S.; Solomonov, A. V.; Timin, A. S.; Rumyantsev, E. V. Recent Advances of Individual BODIPY and BODIPY-Based Functional Materials in Medical Diagnostics and Treatment. *Curr. Med. Chem.* **2017**, *24*, 2745−2772. (c) Kolemen, S.; Akkaya, E. U. Reaction-based BODIPY probes for selective bio-imaging. *Coord. Chem. Rev.* **2018**, *354*, 121−134. (d) Kowada, T.; Maeda, H.; Kikuchi, K. BODIPY-based probes for the fluorescence imaging of biomolecules in living cells. *Chem. Soc. Rev.* **2015**, *44*, 4953−4972. (e) Ni, Y.; Wu, J. Far-red and near infrared BODIPY dyes: synthesis and applications for fluorescent pH probes and bio-imaging. *Org. Biomol. Chem.* **2014**, *12*, 3774−3791. (f) Shi, Z.; Han, X.; Hu, W.; Bai, H.; Peng, B.; Ji, L.; Fan, Q.; Li, L.; Huang, W. Bioapplications of small molecule Aza-BODIPY: from rational structural design to in vivo investigations. *Chem. Soc. Rev.* **2020**, *49*, 7533−7567.

(14) Nokubi, T.; Nasu, K.; Watanabe, R.; Kamimura, A. A New Water-soluble Fluorescent Dye Based on 2-Sulfanyl-1,4-dihydroquinone Dimers. *Chem. Lett.* **2013**, *42*, 876−878.

(15) Kamimura, A.; Nokubi, T.; Watanabe, R.; Ishikawa, M.; Nasu, K.; Uno, H.; Sumimoto, M. 4,4′-Disulfanyl-2,2′,5,5′-tetraoxybiaryl Derivatives as a Water Soluble Fluorescent Dye. *J. Org. Chem.* **2014**, *79*, 1068−1083. Kamimura, A.; Takechi, Y.; Watanabe, R. Preparation of Hydrophobic 2-Phenylthiohydroquinone Dimers and Evaluation of Their Photophysical Properties. *Heteroat. Chem.* **2014**, *25*, 402−409.

(16) Kamimura, A.; Sakamoto, S.; Umemoto, H.; Kawamoto, T.; Sumimoto, M. 2-Sulfanylhydroquinone dimer as a switchable fluorescent dye. *Chem.—Eur. J.* **2019**, *25*, 14081−14088.

(17) The DMSO solution of the dye is stable and does not lose fluorescence activity for a couple of months. The fluorescence activity is also good in pH 7 aqueous solution. The dye is solid and easy to store at room temperature.

(18) Arakawa, T.; Kita, Y.; Timasheff, S. N. Protein precipitation and denaturation by dimethyl sulfoxide. *Biophys. Chem.* **2007**, *131*, 62−70.