Polymeric Self-Assemblies with Boron-Containing Near-Infrared Dye Dimers for Photoacoustic Imaging Probes

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1. Experimental section

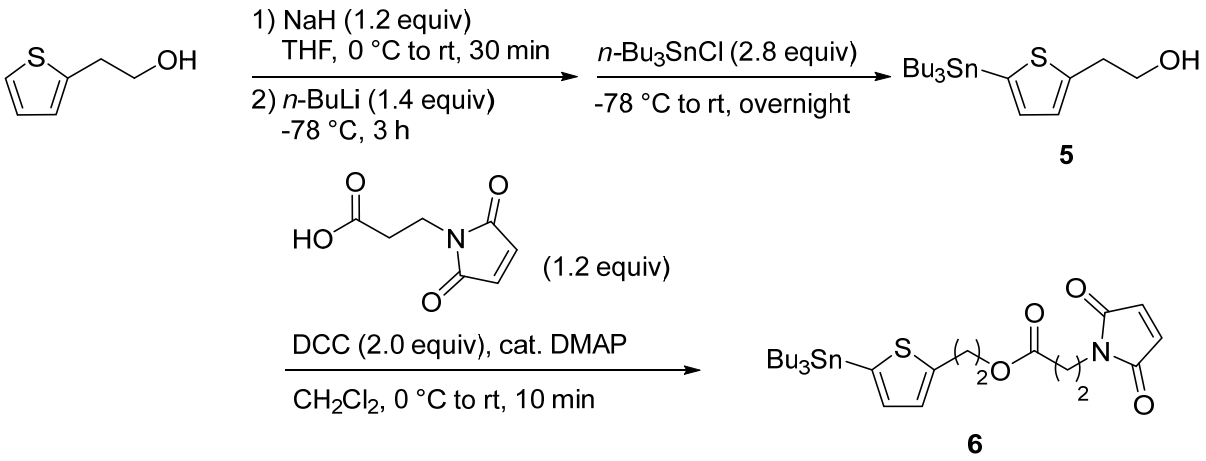
1.1. Materials and Methods

Sodium hydride (60 wt% in oil), *n*-butyl lithium (*n*-BuLi, 1.6 M in hexane), tetrahydrofuran (THF), methanol (MeOH), *N*,*N*-diisopropylethylamine, copper(I) iodide (CuI), 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), potassium carbonate (K_2CO_3), and ethyl acetate (EtOAc) were purchased from nacalai tesque (Japan). Dichloromethane (CH_2Cl_2) was purchased from Kishida Chemical Co., Ltd. (Japan). 2-Thiophenethanol and propargyl bromide were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). *N*,*N'*-Dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were purchased from Watanabe Chemical Industries, Ltd. (Japan). Tributyltin chloride, chloroform ($CHCl_3$), *N*,*N*-dimethylformamide (DMF), and *N*,*N*-diisopropylethylamine were purchased from Wako Pure Chemicals Industries, Ltd. (Japan). All solvents for chemical reactions were distilled over appropriate drying agents before use. Dialysis membrane, Spectra/Por 6 (molecular weight cutoff (MWCO): 25000) were purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA).

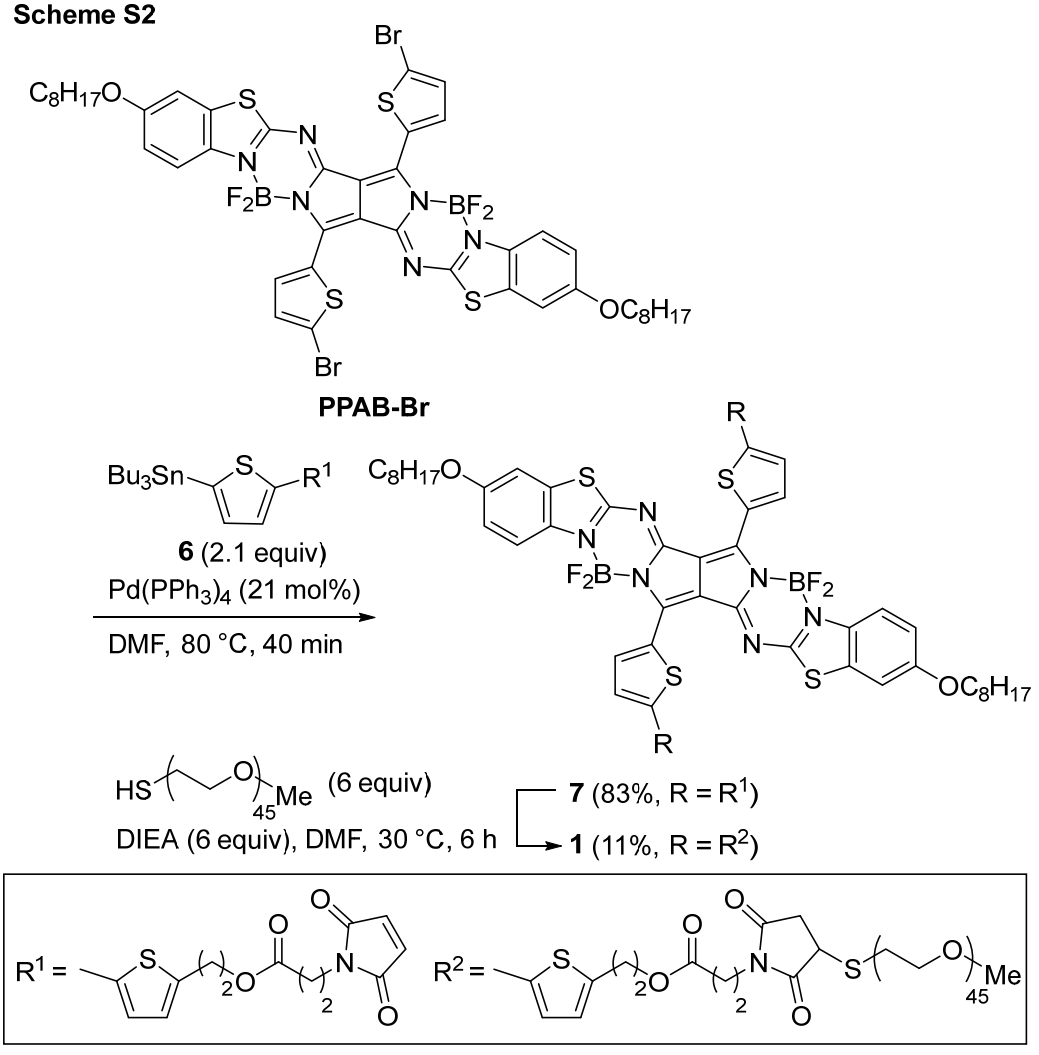
Poly(ethylene glycol) monomethyl ether derivatives having a mercapto group ($CH_3(OCH_2CH_2)_{45}SH$), that having an azido group ($CH_3(OCH_2CH_2)_{45}N_3$), and that having a propargyl group ($CH_3(OCH_2CH_2)_{45}CH_2C{\equiv}CH$) were prepared according to the reported procedure.[1]

1.2. Synthesis of PEGylated PPAB 1

Scheme S1



Scheme S2



1.2.1. Synthesis of thiophene derivative 6

Thiophene derivative **5** was synthesized through the stannylation of 2-thiophenethanol followed by the

condensation reaction. The stannylation was conducted according to the reported procedure.[2] Briefly, to a solution of 2-thiophenethanol (0.55 mL, 5.0 mmol) in THF (10 mL) in a flame-dried Schlenk tube was added sodium hydride (0.24 g, 6.0 mmol) at 0 °C. After stirring for 30 min at room temperature, to this mixture was added *n*-BuLi (4.4 mL, 7.0 mmol) at -78 °C. After stirring for 3 h, to this mixture was added tributyltin chloride (3.8 mL, 14 mmol) at -78 °C. After stirring overnight at room temperature, the mixture was filtered through Celite pad. The organic layer was successively washed with sat. NH_4Cl aqueous solution (10 mL×2) and brine (10 mL×2). The organic layer was dried over Na_2SO_4. The organic solvents were removed under reduced pressure and the residue was subjected to column chromatography on SiO_2 with hexane and EtOAc (v:v = 1:0 to 4:1) as eluents to afford **5** (0.41 g, 0.98 mmol, 19%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 0.71-0.80 (m, 9H), 0.87-1.17 (m, 6H), 1.24-1.50 (m, 6H), 1.52-1.68 (m, 6H), 2.04 (s, 1H), 3.13 (t, *J* = 6.1 Hz, 2H), 3.87 (t, *J* = 6.1 Hz, 2H), 7.0 (d, *J* = 2.9 Hz, 1H), 7.0 (d, *J* = 2.9 Hz, 1H).

To a solution of 3-maleimidopropionic acid[3] (0.20 g, 1.2 mmol) in CH_2Cl_2 (10 mL) in a flame-dried Schlenk tube were added **5** (0.42 g, 1.0 mmol), DCC (0.41 g, 2.0 mmol), and DMAP (13 mg, 0.10 mmol) at 0 °C. After stirring for 30 min at room temperature, the organic solvent was removed under reduced pressure and the residue was subjected to column chromatography on SiO_2 with hexane and EtOAc (v:v = 1:0 to 5:1) as eluents to afford **6** (0.39 g, 0.68 mmol, 68%) as a yellow oil. IR (ATR) 2956, 2923, 2871, 2854, 1739, 1712 (C=O), 1657 (C=C), 1609, 1456, 1408, 1376, 1340, 1293, 1252, 1179, 1074, 1046, 960, 934, 876, 826, 799, 769, 744 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C) δ 0.89 (t, *J* = 7.3 Hz, 9H), 1.06-1.11 (m, 6H), 1.27-1.39 (m, 6H), 1.51-1.59 (m, 6H), 2.66 (t, *J* = 7.1 Hz, 2H), 3.18 (t, *J* = 7.0 Hz, 2H), 3.83 (t, *J* = 7.1 Hz, 2H), 4.31 (t, *J* = 7.1 Hz, 2H), 6.97 (d, *J* = 3.3 Hz, 1H), 7.00 (d, *J* = 3.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 10.7, 13.7, 27.2, 28.9, 32.9, 33.6, 65.1, 99.9, 126.8, 134.2, 135.3, 170.3, 170.6. HRMS (FAB) calcd for $C_{25}H_{39}NO_4SSn$ (M^+): 569.1622, found: 569.1631.

1.2.2. Synthesis of PEGylated PPAB 1

To a solution of **PPAB-Br**[4] (19 mg, 1.8×10^{-2} mmol) and **6** (22 mg, 3.9×10^{-2} mmol) in DMF (3 mL) in a flame-dried Schlenk tube was added $Pd(PPh_3)_4$ (4.4 mg, 3.8 μmol) at room temperature. After stirring for 40 min at

80 °C, the reaction mixture was cooled to room temperature. The mixture was filtered through short Florisil pad and the filtrate was diluted by water (20 mL). The precipitate was separated by filtration using Celite pad and washed with water (8 mL) and MeOH (8 mL). The precipitate was collected by dissolving in $CHCl_3$. The organic solvent was removed under reduced pressure and the residue was purified by gel permeation chromatography with $CHCl_3$ as an eluent to afford **7** (22 mg, 1.5×10^{-2} mmol, 83%) as a dark purple solid. mp >300 °C; IR (neat) 2962, 2925, 2852, 1704 (C=O), 1455, 1418, 1263, 1184, 1057, 799 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 25 °C) δ 0.87-1.08 (m, 6H), 1.25-1.37 (m, 12H), 1.42-1.52 (m, 4H), 1.54-1.65 (m, 4H), 1.79-1.85 (m, 4H), 2.71 (t, *J* = 6.9 Hz, 4H), 3.16 (t, *J* = 6.9 Hz, 4H), 3.86 (t, *J* = 7.0 Hz, 4H), 3.97 (t, *J* = 6.4 Hz, 4H), 4.34 (t, *J* = 6.6 Hz, 4H), 6.69 (s, 4H), 6.81 (s, 2H), 7.03 (d, *J* = 8.9 Hz, 2H), 7.07 (br, 2H), 7.22 (d, *J* = 4.0 Hz, 2H), 7.25 (d, *J* = 3.4 Hz, 2H), 7.89 (d, *J* = 8.6 Hz, 2H), 9.08 (d, *J* = 4.3 Hz, 2H). HRMS (FAB) calcd for $C_{70}H_{69}B_2F_4N_8O_{10}S_6$ ($M+H^+$): 1471.3605, found: 1471.3596.

To a solution of **7** (3.1 mg, 2.1 μmol) and *N*,*N*-diisopropylethylamine (2.2 μL, 1.3×10^{-2} mmol) in DMF (1 mL) in a flame-dried Schlenk tube was added $CH_3(OCH_2CH_2)_{45}SH$ (MW = 2000, 26 mg, 1.3×10^{-2} mmol) at room temperature. After stirring for 6 h at 30 °C, the reaction mixture was diluted by water (2 mL). The mixture was dialyzed against H_2O for 1 day by using Spectra/Por 6 (MWCO = 25000). The PEGylated PPAB **1** (1.3 mg, 11%) was obtained as a purple solid after lyophilization. ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ 0.88-0.90 (m, 20H), 1.31 (m, 68H), 1.83 (m, 11H), 2.55 (m, 6H), 2.74 (m, 6H), 3.38 (s, 14H), 3.47-3.82 (m, 830H), 4.03 (s, 8H), 5.30 (s, 8H), 7.09 (br, 4H), 7.20 (s, 2H), 7.35 (d, *J* = 3.4 Hz, 2H), 7.38 (d, *J* = 4.4 Hz, 2H), 7.98 (d, *J* = 9.8 Hz, 2H), 9.17 (d, *J* = 3.4 Hz, 2H).

1.3. Synthesis of PEGylated TBD 3

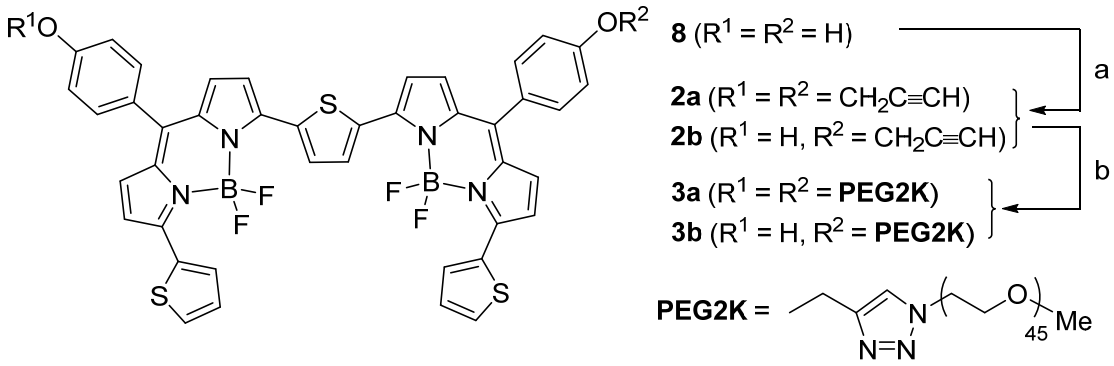


Figure S1. Synthesis of PEGylated TBD derivatives **3**. Conditions: (a) NaH, propargyl bromide, THF; (b) $CH_3(OCH_2CH_2)_{45}N_3$, CuI, DBU, DMF.

1.3.1. Synthesis of TBD derivatives 2 having propargyl groups

TBD derivatives having propargyl groups were synthesized from the known TBD derivative **8**.[5] **8** (22.6 mg, 27.8 μmol) and K_2CO_3 (15.4 mg, 111 μmol) were added to 2.5 mL of acetone under Ar atmosphere, and stirred at room temperature. Then propargyl bromide (4.2 μL, 56 μmol) was added to the reaction mixture, which was stirred at 50 °C for 27 h. The solvent was removed in vacuo. The resultant solid was purified by silica-gel column chromatography (Kanto silica gel 63–210 μm, eluent: dichloromethane/acetone = 1:0 ~ 1:1), and reprecipitation from dichloromethane/hexane gave **2a** (14.6 mg, 16.4 μmol, 59%) and **2b** (5.3 mg, 5.9 μmol, 21%).

2a: green solid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.61 (t, J = 2.4 Hz, 2H), 4.81 (d, J = 2.4 Hz, 4H), 6.84 (d, J = 4.4 Hz, 2H), 6.84–6.86 (m, 4H), 6.92 (d, J = 4.4 Hz, 2H), 7.13 (d, J = 8.6 Hz, 4H), 7.24 (m, 2H), 7.51–7.53 (m, 6H), 8.27 (dd, J = 1.2 Hz, 4.0 Hz, 2H), 8.28 (s, 2H); ^{11}B NMR (128 MHz, $CDCl_3$) δ 1.74 (t, J = 33.0 Hz). ^{19}F NMR (376 MHz, $CDCl_3$) δ –139.5 (q, J = 33.0 Hz). Anal. Calcd for $C_{48}H_{30}B_2F_4N_4O_2S_3 \cdot 0.3H_2O$: C, 64.49; H, 3.45; N, 6.27. Found: C, 64.30; H, 3.61; N, 6.21. MALDI-TOF MS for $C_{48}H_{30}B_2F_4N_4O_2S_3$ $[M]^+$: m/z = 888.2; found: 888.3.

2b: green solid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.61 (t, J = 2.4 Hz, 1H), 4.81 (d, J = 2.4 Hz, 2H), 6.84–6.86 (m, 6H), 6.92 (d, J = 4.4 Hz, 2H), 6.98 (d, J = 8.6 Hz, 2H), 7.13 (d, J = 8.6 Hz, 2H), 7.23–7.25 (m, 2H), 7.45 (d, J = 8.6 Hz, 2H), 7.51–7.53 (m, 4H), 8.26-8.29 (m, 4H); ^{11}B NMR (128 MHz, $CDCl_3$) δ 1.74 (t, J = 33.0 Hz). ^{19}F

NMR (376 MHz, $CDCl_3$) δ –139.5 (q, J = 33.0 Hz). HRMS (ESI) for $C_{45}H_{28}B_2F_4N_4O_2S_3Na$ $[M{\cdot}Na]^+$: m/z = 873.1389; found: 873.1379.

1.3.2. Synthesis of PEGylated TBD derivatives 3

A solution of **2b** (3.2 mg, 3.7 μmol), $CH_3(OCH_2CH_2)_{45}N_3$ (7.5 mg, 3.8 μmol), CuI (71 μg, 0.37 μmol), and DBU (0.056 μL, 0.37μmol) in DMF (3 mL) in a flame-dried Schlenk tube was heated at 60 °C overnight. The reaction mixture was cooled to room temperature and diluted by water (2 mL). The mixture was dialyzed against H_2O for 1 day by using Spectra/Por 6 (MWCO = 25000). The PEGylated TBD **3b** (9.5 mg, 67%) was obtained as a purple solid after lyophilization. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 3.38 (s, 3H), 3.46 (t, J = 4.6 Hz, 2H), 3.54-3.75 (m, 335H), 3.75-3.85 (m, 2H), 3.85-4.00 (m, 2H), 4.60 (s, 2H), 5.30 (br s, 2H), 6.80-6.96 (m, 8H), 7.10-7.25 (m, 4H), 7.38-7.55 (m, 8H), 7.94 (br s, 1H), 8.24-8.34 (m, 4H).

PEGylated TBD **3a** was similarly prepared from **2a**. **3a**: pale purple solid (64% yield); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 3.38 (s, 6H), 3.46 (t, J = 4.9 Hz, 4H), 3.54-3.77 (br, 406H), 3.82 (t, J = 4.7 Hz, 4H), 3.92 (t, J = 4.9 Hz, 4H), 4.60 (t, J = 4.9 Hz, 4H), 5.31 (s, 4H), 6.82-6.87 (m, 6H), 6.92 (d, J = 4.4 Hz, 2H), 7.16 (d, J = 8.8 Hz, 4H), 7.50-7.53 (m, 6H), 7.94 (s, 2H), 8.27 (d, J = 5.8 Hz, 4H).

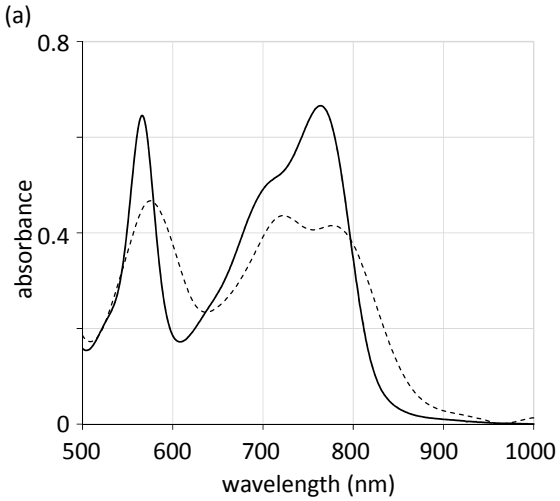


Figure S2. (a) Absorption spectra of **3b** (0.1 mg/mL) in DMF (solid) and H_2O (dashed). (b) Photoluminescence spectra of **3b** in DMF and H_2O (λ_{ex} = 720 nm).

1.4. Synthesis of TBD-grafted HA derivatives 4

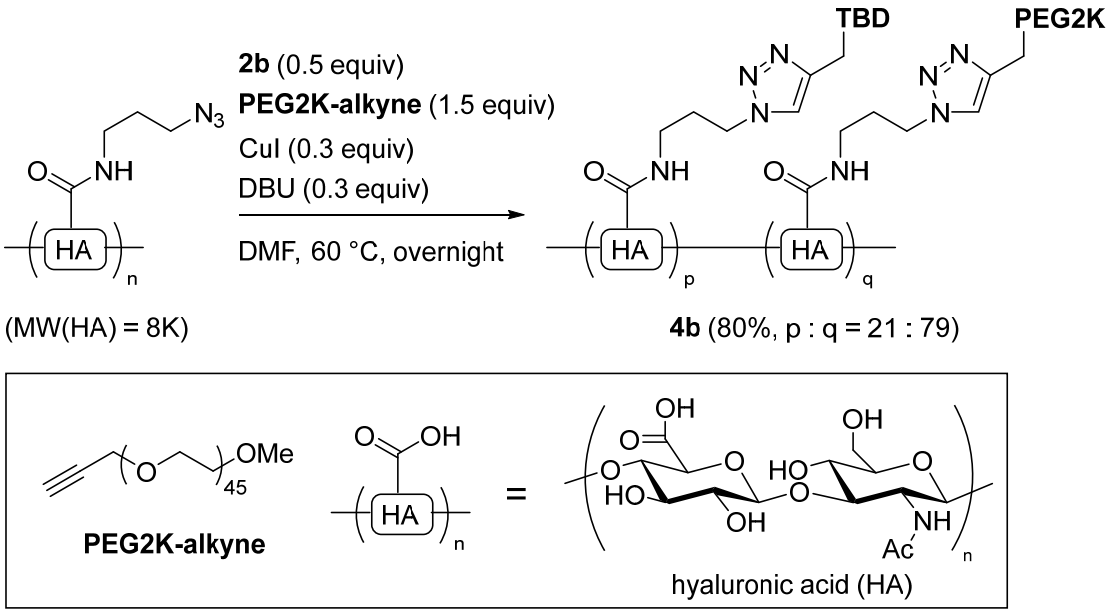


Figure S3. Synthesis of TBD-grafted HA derivative **4b**.

TBD-grafted HA derivatives **4** were prepared according to the reported procedure.[6] Briefly, a solution of hyaluronic acid derivative having azide groups at the side chain terminals (3.5 mg, MW(HA) = 8K, 0.35 μmol of polymer involving 7.5 μmol of the repeating unit), **2b** (3.5 mg, 4.1 μmol), $CH_3(OCH_2CH_2)_{45}CH_2C{\equiv}CH$ (23 mg, 11 μmol), CuI (0.43 mg, 2.2 μmol), and DBU (0.33 μL, 2.2 μmol) in dry DMF (3.0 mL) was heated at 60 °C overnight. The reaction mixture was cooled to room temperature and diluted by water (2 mL). The mixture was dialyzed against H_2O for 1 day by using Spectra/ Por 6 (MWCO = 25000). TBD-grafted HA derivatives **4b** (13 mg, 89%) was obtained as a purple solid after lyophilization. The ^{1}H NMR spectrum shows that azido groups were completely converted to triazoles. The grafting efficiency of TBD moieties into **4b** was determined by comparing UV−vis absorbance of **4b** with that of **2b** as a standard compound at 760 nm. ^{1}H NMR (500 MHz, D_2O, 25 °C) δ 1.80–2.01 (m, 3H), 3.23 (br s, 29H), 3.23-4.00 (m, 3H), 4.32-4.40 (m, 4H), 7.90 (br s, 1H).

TBD-grafted HA derivative **4a** was similarly prepared from **2a**. **4a**: purple solid (26% yield); ^{1}H NMR (500 MHz, D_2O, 25 °C) δ 1.86-2.02 (m, 3H), 3.23 (br s, 82H), 3.35-4.00 (m, 4819H), 4.02-4.20 (br s, 40H), 7.91 (br s, 1.03H).

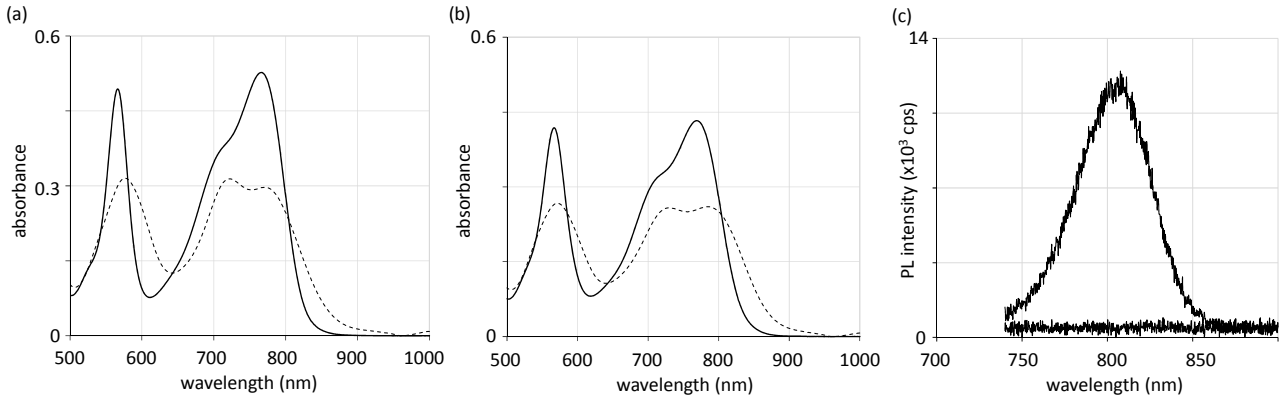


Figure S4. Absorption spectra of (a) **4a** and (b) **4b** (0.1 mg/mL) in DMF (solid) and H_2O (dashed). (c) Photoluminescence spectra of **4b** in DMF and H_2O (λ_{ex} = 720 nm).

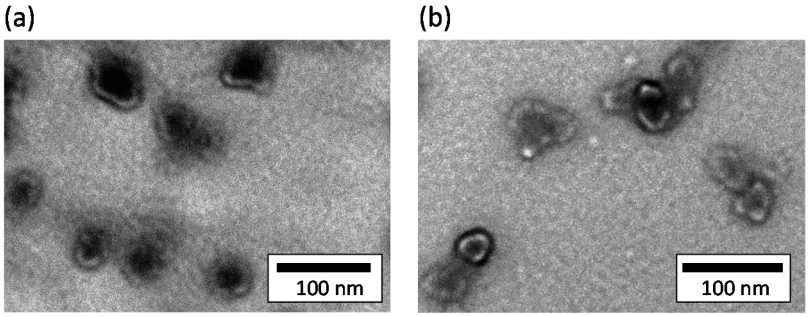


Figure S5. TEM images of (a) **4a** and (b) **4b**.

1.5. Fluorescence intensities of dye-conjugated amphiphiles

Fluorescence intensities of PPAB- and TBD-conjugated amphiphiles **1**, **3**, and **4** in DMF and water (0.50~5.0 nmol (dye)/5.0 μL) were measured by IVIS Imaging System 200 Series (Perkin Elmer, λ_{exc} = 745 nm, λ_{em} = 820 nm). Fluorescence intensities were quantified by Living Image 2.50-Igor Pro 4.09 software (Perkin Elmer). Although strong fluorescence were detected from DMF solutions, no fluorescence emission were observed from aqueous solutions.

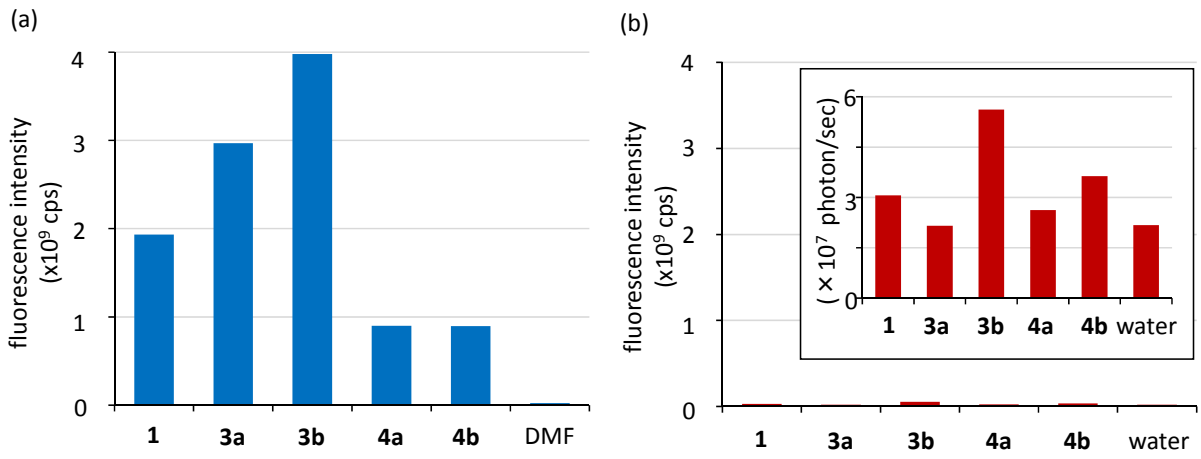


Figure S6. Fluorescence intensity of dye-conjugated amphiphiles in DMF (left) and water (right).

1.6. Photoacoustic signals of dye-conjugated amphiphiles

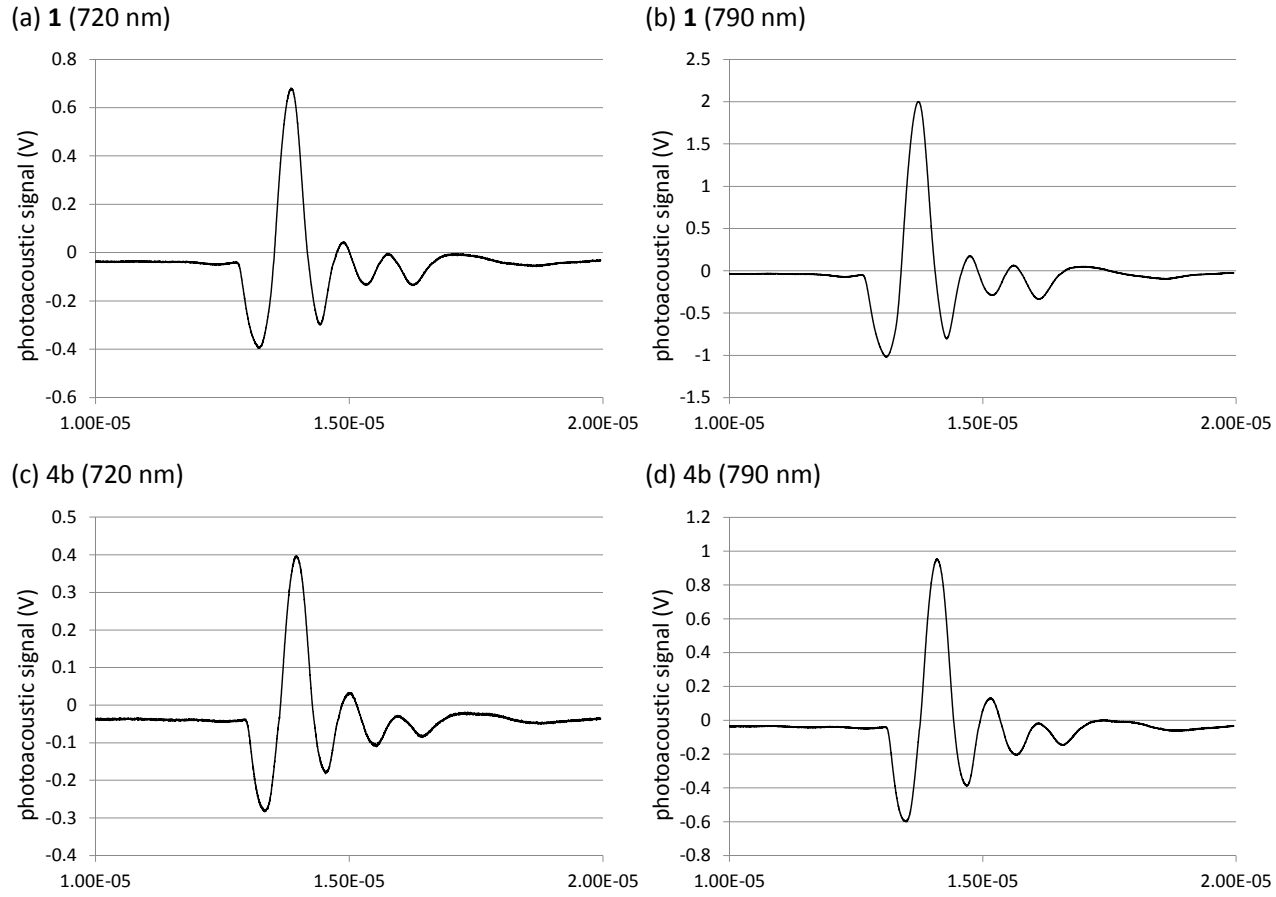


Figure S7. Representative photoacoustic signals of **1** irradiated at (a) 720 nm and (b) 790 nm, and **4b** irradiated at (c) 720 nm and (d) 790 nm.

1.7. Measurement of cac

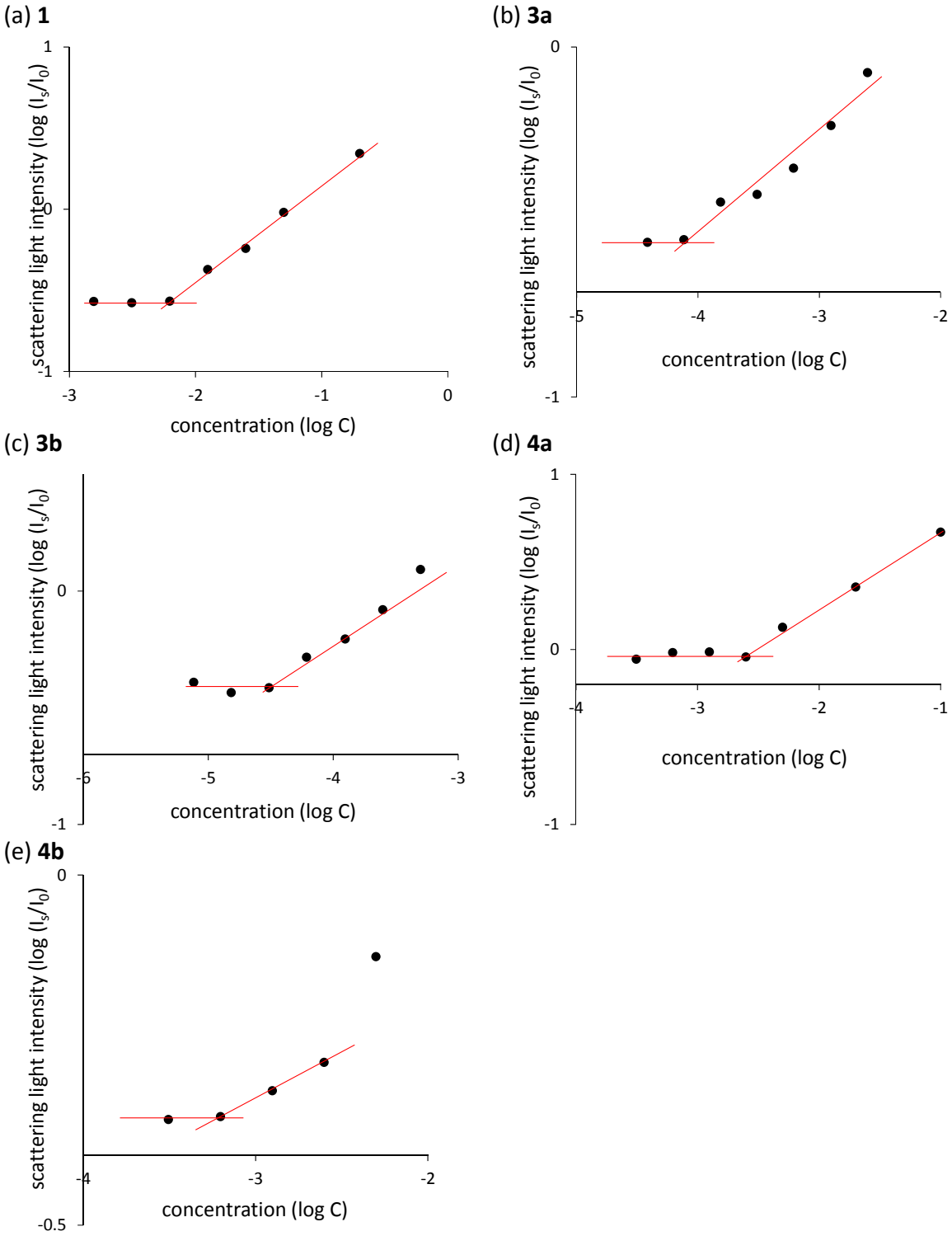


Figure S8. Static light scattering measurements of self-assemblies.

1.8. Photobleaching test

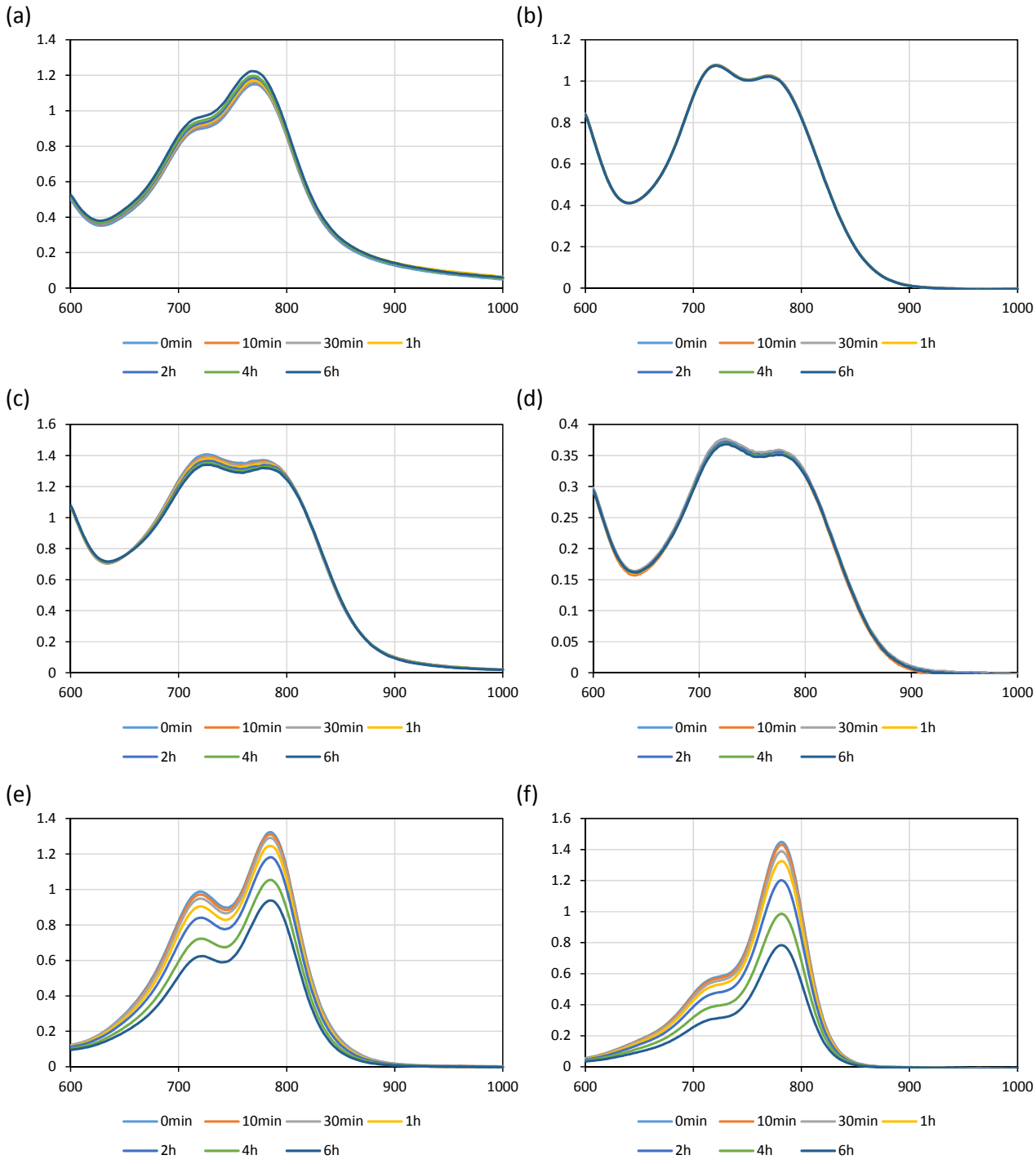


Figure S9. Time-dependent change of absorbance spectra of (a) **1**, (b) **3a**, (c) **4a**, (d) **4b**, (e) **5**, and (f) indocyanine green in DMF/H_2O (v:v = 1:20) after continuous photoirradiation (12 μW·cm^{-2}, visible light).

1.9. Stability tests of 4b under physiological conditions

The absorbance and PA signals of TBD-conjugated HA derivative **4b** in PBS (pH 7.4) and 75% FCS solutions before and after 24 h incubation were measured. The absorbance spectra of **4b** were not changed after 24 h incubation (Figures S10 and S11a). Furthermore, the changes of PA signal intensities of **4b** (λ_{exc} = 790 nm) in PBS and FCS solutions were negligible after 24 h (Figure S11b).

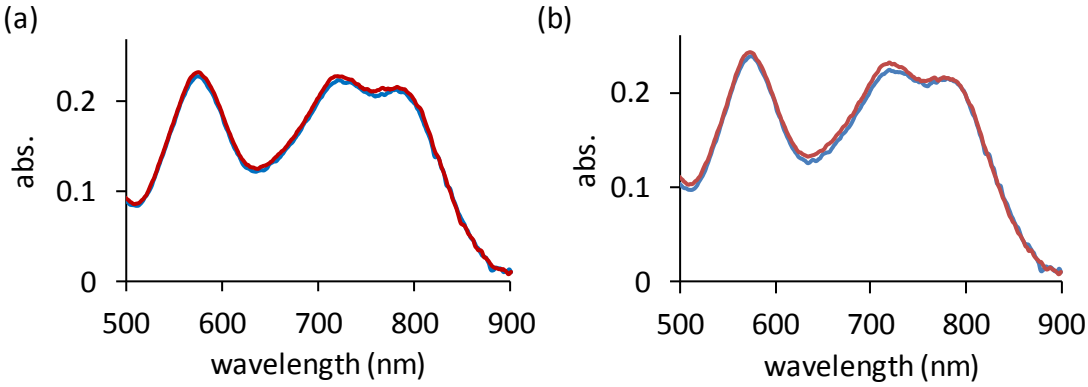


Figure S10. UV-vis spectra of **4b** in 0 h (red) and 24 h (blue) in (a) PBS and (b) FCS.

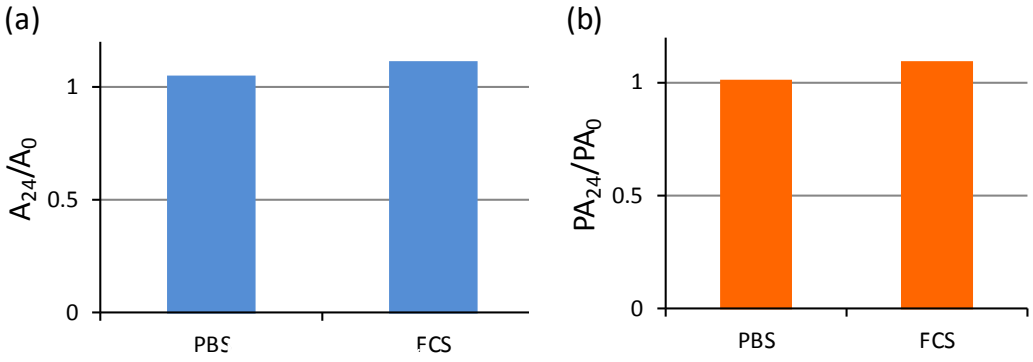


Figure S11. Fold change of (a) absorbance (λ_{abs} = 790 nm) and (b) PA signal intensity of **4b** (λ_{exc} = 790 nm) in PBS and FCS (24 h/0 h).

1.10. Delivery of dye-grafted HA derivative to cells

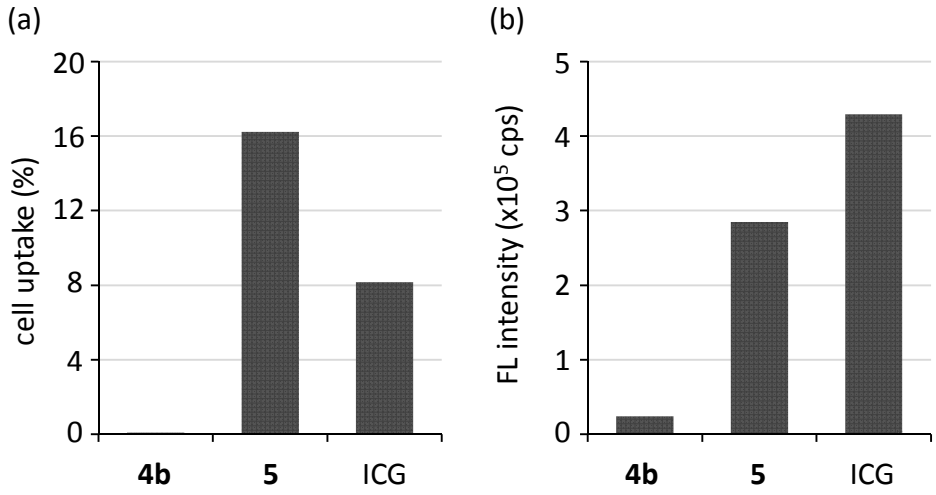


Figure S12. (a) Cell uptake ratio of **4b**, **5**, and ICG determined by fluorescence intensities. (b) Fluorescence intensity of cells 24 h after incubation.

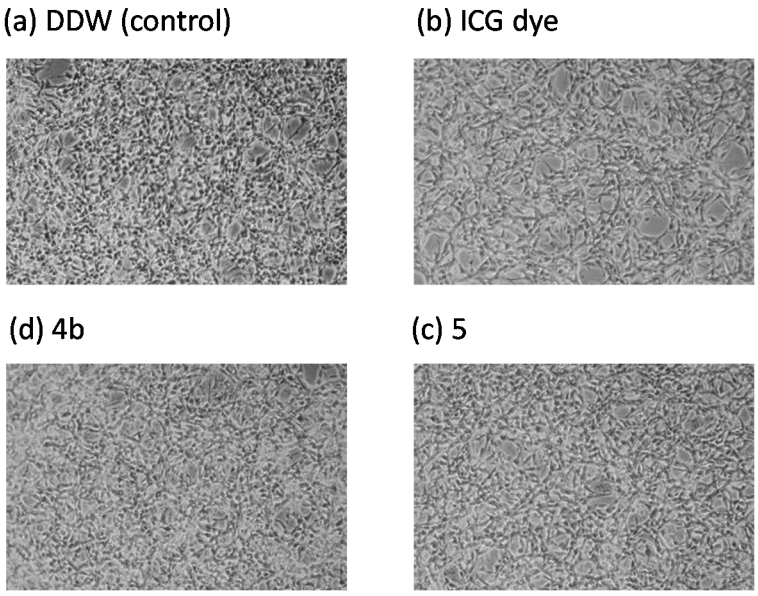


Figure S13. Images of colon26 cells culture (a) without dyes or with (b) ICG dye, (c) **4b**, and (d) **5** measured by phase contrast light microscope

2. References

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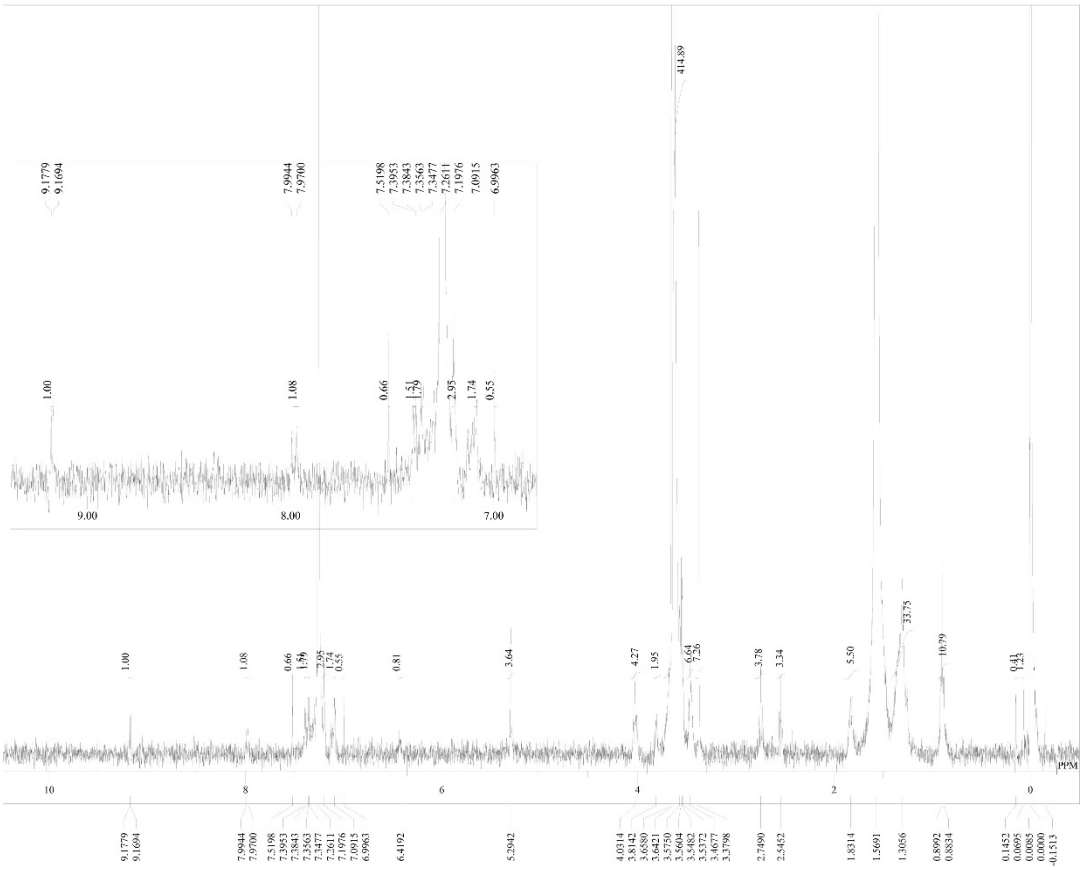
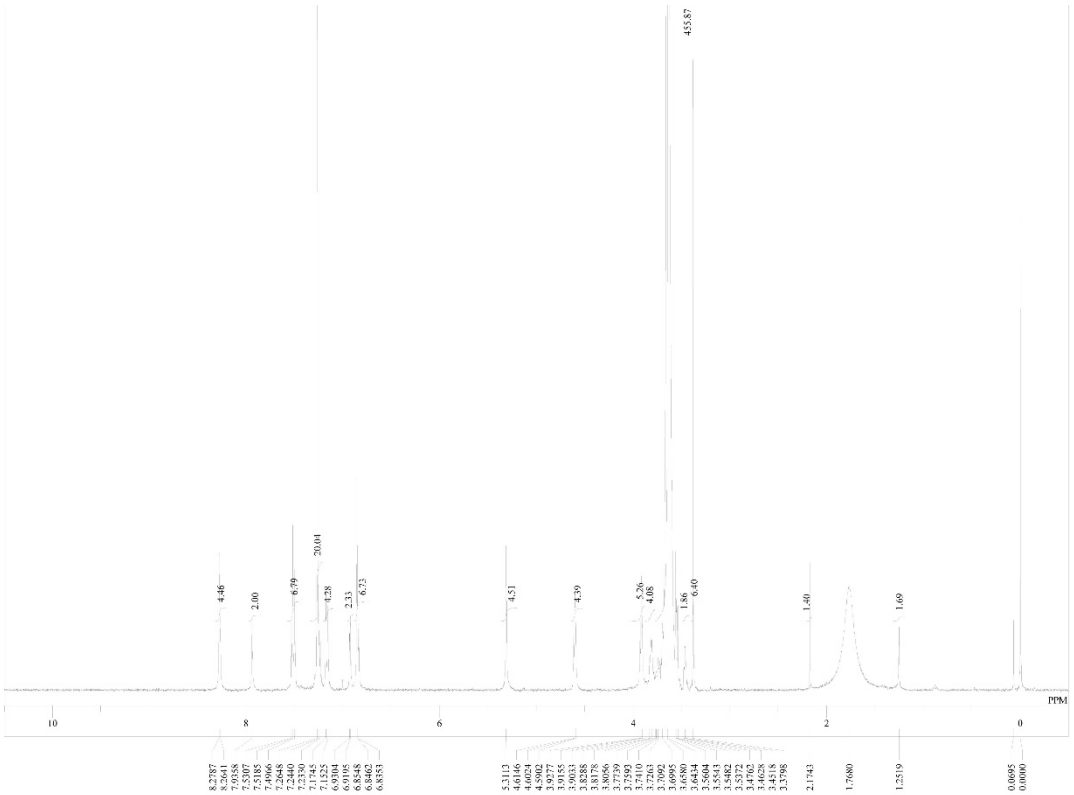
3. NMR spectra

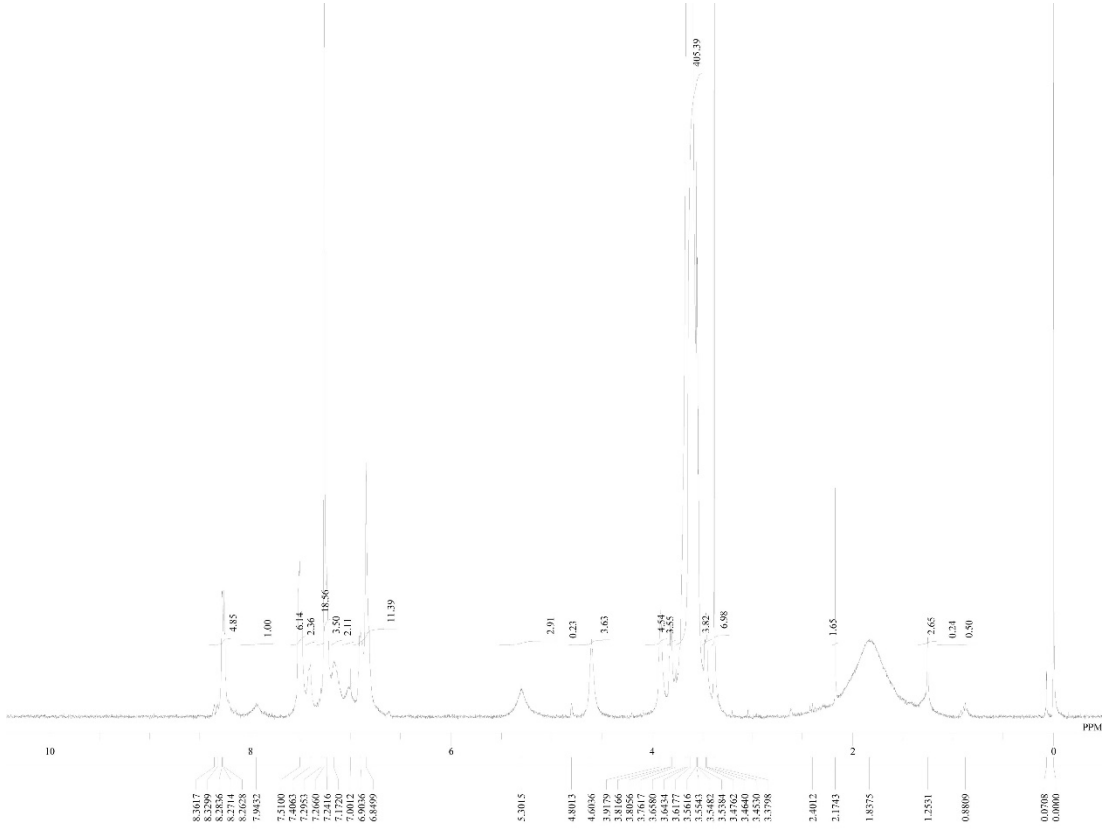
^{1}H NMR spectrum of **1**



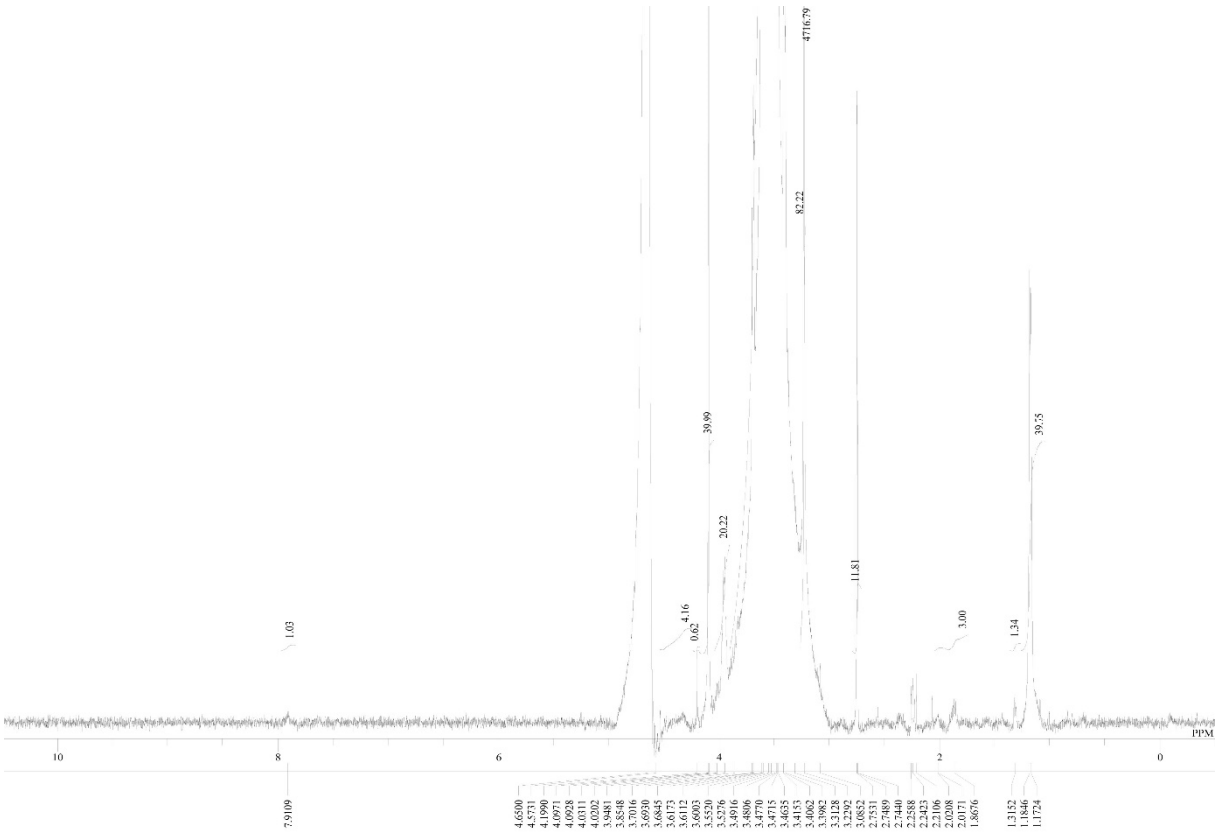
^{1}H NMR spectrum of **3a**



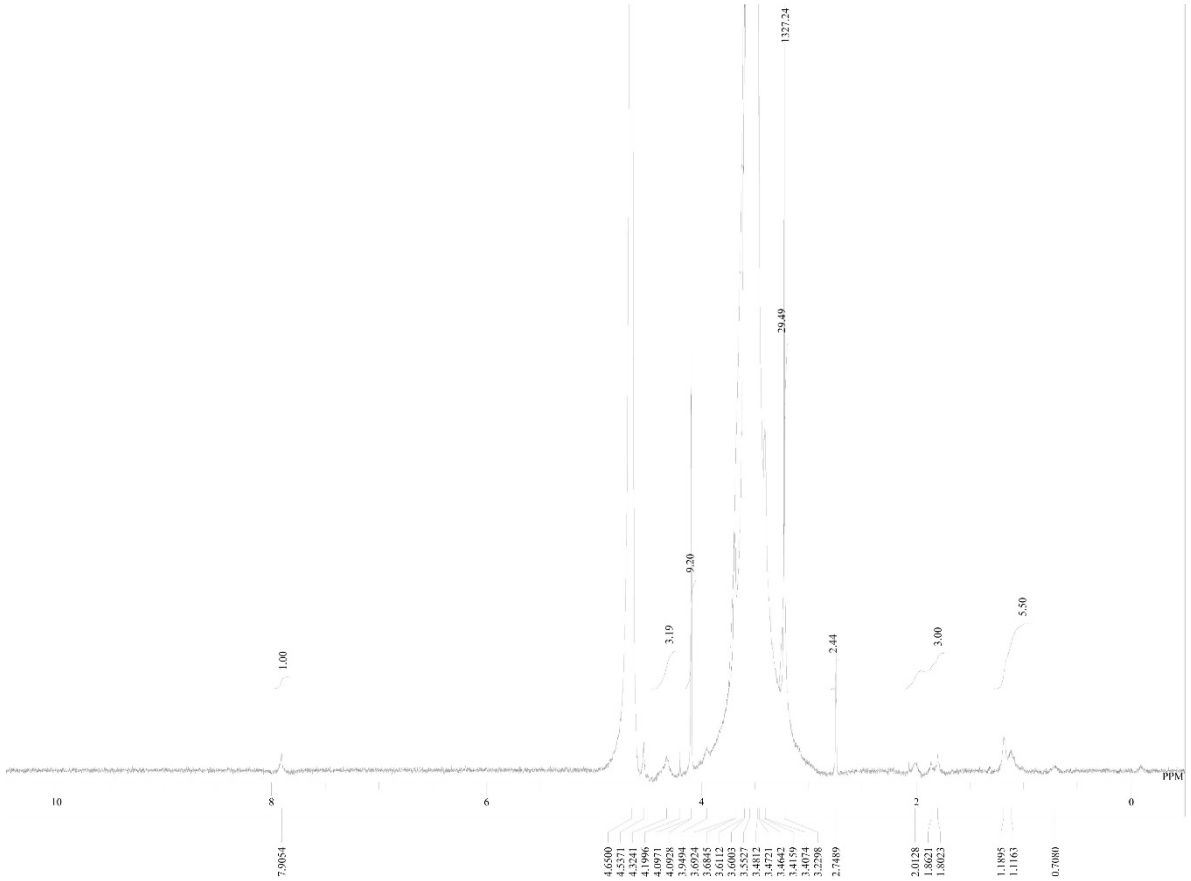
^{1}H NMR spectrum of **3b**



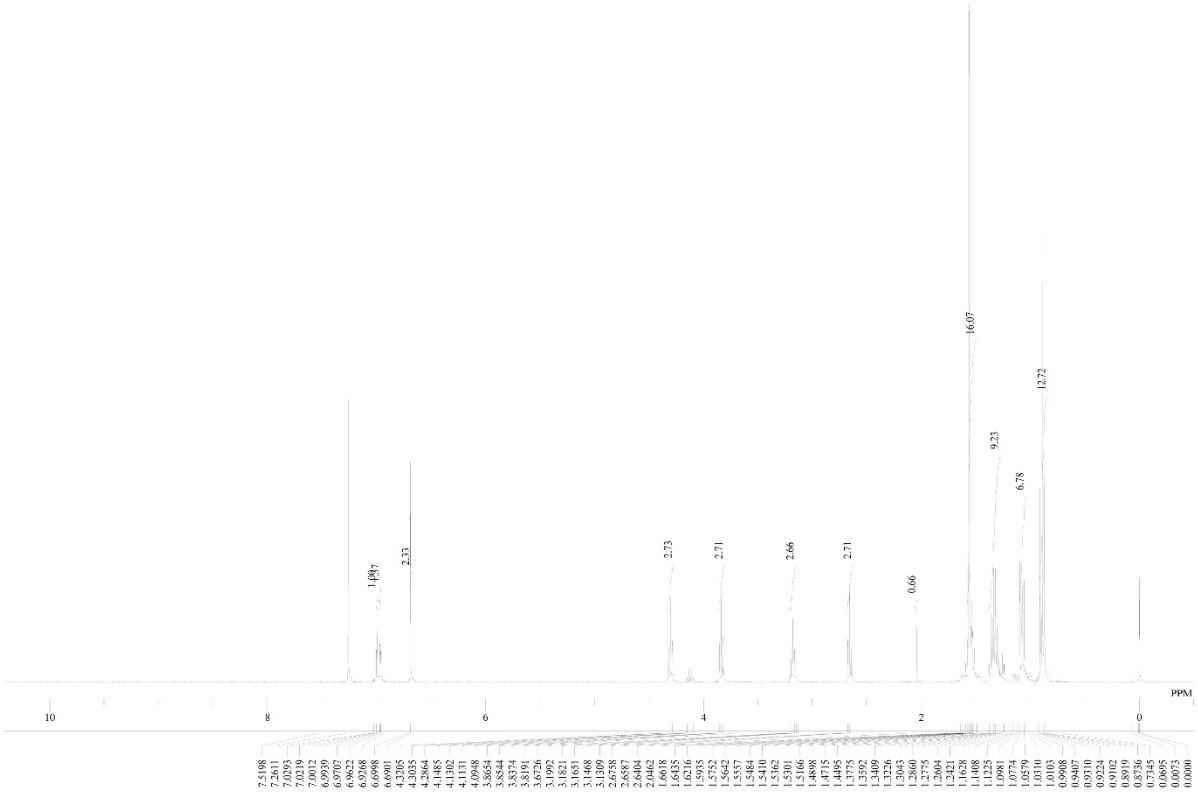
^{1}H NMR spectrum of **4a**



^{1}H NMR spectrum of **4b**



^{1}H NMR spectrum of **6**



^{1}H NMR spectrum of **7**