Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Water-soluble Oligofluorenes bearing N_1-Alkylcytosine Side Chain as Turn-on and Turn-off Materials in Telomere DNA Length Sensing

Isao Yamaguchi, Ryoga Ooe and Aohan Wang

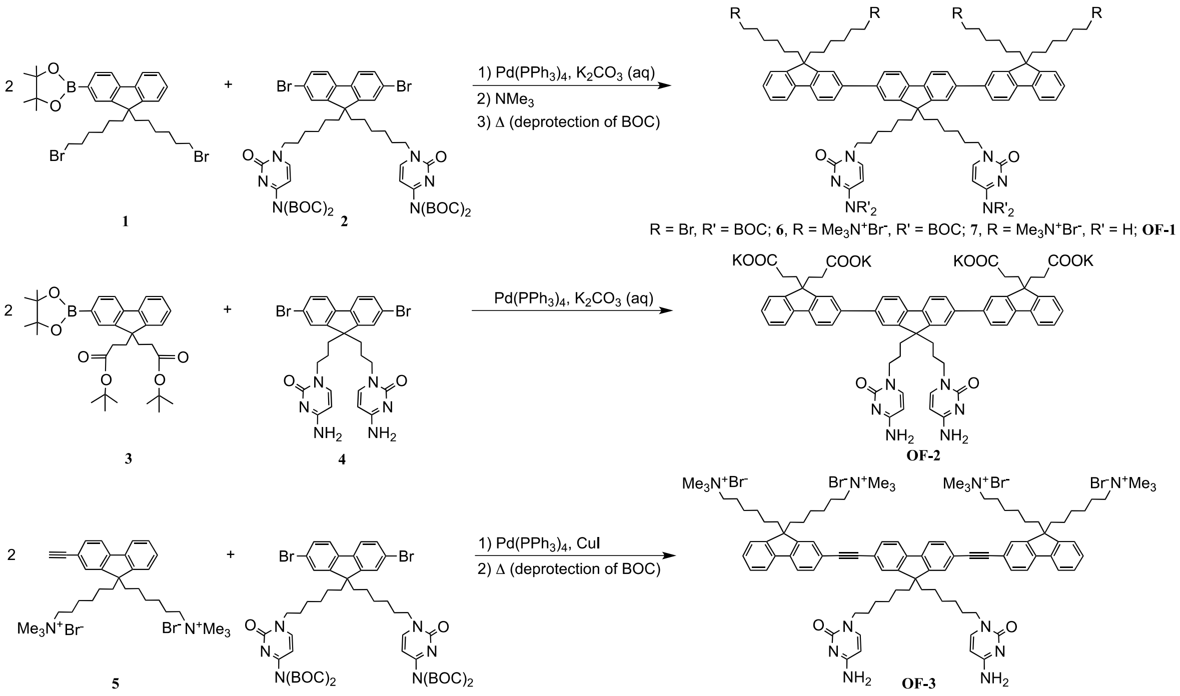
1. Methods

1.1. General Methods. Solvents were dried, distilled, and stored under nitrogen. 9,9-Bis(6-bromohexyl)fluorene-2-boronic acid pinacol ester (**1**),[1] 2,7-dibromo-9,9-bis(6-(*N*,*N*-bis-*tert*-butoxycarbonyl)cytosylhexyl)fluorene (**2**),[2] 2,7-dibromo-9,9-bis(3-bromopropyl)fluorene[3] and 2-bromo-9,9-bis(3-*tert*-butylpropanoate)fluorene[4] were synthesized according to the literatures. $(TTAGGG)_m$ purified by HPLC were purchased from Nippon Gene Co. LTD. Other reagents were purchased and used without further purification. Reactions were carried out with standard Schlenk techniques under nitrogen.

IR and NMR spectra were recorded on a JASCO FT/IR-660 PLUS spectrophotometer with a KBr pellet and JEOL AL-400 and JEOL ECX-500 spectrometers, respectively. UV-vis and PL spectra were obtained by a JASCO V-560 spectrometer and a JASCO FP-6200, respectively. Quantum yields were calculated by using a diluted ethanol solution of 7-dimethylamino-4-methylcoumarin as the standard. Fluorescence lifetime measurements were conducted with a HORIBA FluoroCube Model1000U. **OF-1** and **OF-2** were excited with 370 nm and 340 nm diode lasers (Horiba NanoLED), respectively. Aqueous suspension of TM-40 colloidal silica (40wt%) was used for a prompt measurement. Cyclic voltammetry was performed with a Hokuto Denko HSV-110, and 1 cm

× 1 cm and 1 cm × 2 cm Pt plates and Pt wire were used as working, counter and reference electrodes, respectively. Tetraethylammonium tetrafluoroborate was used as an electrolyte. The scan speed was 50 mV•s^{-1}. Electrical conductivity measurements were conducted with a HORIBA Cond Meter ES-51.

1.2. Synthesis



Scheme S1. Synthesis of OFs.

1.2.1. Synthesis of OF-1. Pd(PPh$_3$)$_4$ (0.072 g, 0.062 mmol) was dissolved in 3 mL of dry THF under N$_2$. **1** (0.40 g, 0.65 mmol) and **2** (0.33 g, 0.30 mmol) were added to the solution. After K$_2$CO$_3$(aq) (2.5M, 4 mL; N$_2$ bubbled before use) was added dropwise to the solution, the mixture was refluxed for 48 h. The solvent was removed under vacuum and the resulting solid was dissolved in chloroform

(50 mL). The solution was washed with water and dried over magnesium sulfate. After the solvent was removed under vacuum and the resulting solid was purified by silica gel column chromatography (hexane/EtOAc = 3/7). The solvent was removed under vacuum and the resulting solid was dried under vacuum to give **6** as a yellow powder (0.21 g, 36%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.64-7.82 (14H), 7.33-7.37 (8H), 6.91 (2H), 3.68 (4H), 3.29 (8H), 2.06 (8H), 1.63 (8H), 1.53 (36H), 1.10-1.26 (32H), 0.73 (12H).

6 (0.089 g, 0.0 46 mmol) was dissolved in THF (1 mL). A THF solution of trimethylamine (2 M, 5 mL) was added to the solution. After the reaction solution was stirred at 20 ˚C for 72 h, the precipitate was collected by filtration and dried under vacuum to give **7** as a white powder (0.058 g, 58%). ^{1}H NMR (400 MHz, DMSO-d_6): 7.78-8.09 (14H), 7.78 (2H), 7.51 (6H), 6.72 (2H), 3.71 (4H), 3.15 (12H), 2.96-2.98 (44H), 2.13 (8H), 1.46 (40H), 1.04-1.11 (24H), 0.57-0.66 (12H).

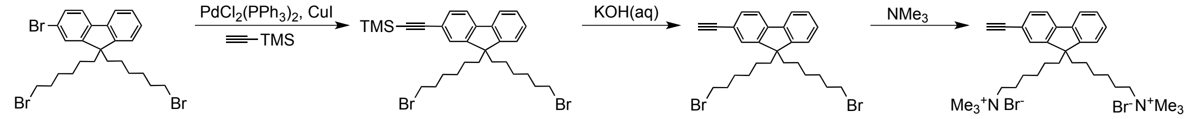
7 was heated at 100 ˚C for 32 h and then at 120 ˚C for 5 h to give **OF-1** as a yellow powder (0.034 g, 55%). ^{1}H NMR (400 MHz, DMSO-d_6): 7.33-7.95 (22H), 5.98 (2H), 3.46 (4H), 3.15 (8H), 2.95 (36H), 3.13 (12H), 1.45 (12H), 1.05 (24H), 0.63 (12H).

1.2.2. Synthesis of 9,9-bis(3-*tert*-butylpropanoate)fluorene-2-boronic acid pinacol ester (3). 2-Bromo-9,9-bis(3-*tert*-butylpropanoate)fluorene[5] (1.0 g, 1.2 mmol) and potassium carbonate (0.75 g, 7.7 mmol) were suspended in 8 ml of dry DMF and then Pd(dppf)Cl_2 (0.048 g, 0.066 mmol) was added to the suspension. After the reaction mixture was stirred at 90 ˚C for 12 h, it was extracted with chloroform. The organic layer was dried over magnesium sulfate and the solvent was removed under vacuum. The resulting solid was purified by silica gel column chromatography (eluent = EtOAc/hexane = 1/1 (*v*/*v*)). The solvents were removed under vacuum and the resulting solid was dried under vacuum to give **3** as a white powder (0.86 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.68-7.83 (4H), 7.33-7.39 (3H), 2.35 (4H), 1.40-1.55 (4H), 1.39 (12H), 1.30 (18H).

1.2.3. Synthesis of 2,7-dibromo-9,9-bis(3-cytosylpropyl)fluorene (4). NaH (0.096 g, 4.0 mmol) was dissolved in dry DMSO (10 mL) at 75 ˚C and then the solution was cooled to 30 ˚C. Cytosine (0.45 g, 4.0 mmol) was added to the solution and the solution was stirred for 1 h, followed by the addition of 2,7-dibromo-9,9-bis(6-bromopropyl)fluorene (0.50 g, 0.89 mmol). After the reaction solution was stirred at 30 ˚C for 20 h, the solvent was removed under vacuum. The resulting solid was washed with $CHCl_3$ (50 mL) and water (50 mL) at 60 ˚C, collected by filtration and dried under vacuum to give **4** as a white solid (0.44 g, 79%). ^{1}H NMR (400 MHz, DMSO-d_6): 7.78 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 1.6 Hz, 2H), 7.53 (d, J = 6.4 Hz, 2H), 7.33 (d, J = 7.2 Hz, 2H), 6.84 and 6.93 (4H), 5.53 (d, J = 6.8 Hz, 2H), 3.38 (t, J = 7.2 Hz, 2H), 1.98 (m, 4H), 0.74 (m, 4H).

1.2.4. Synthesis of OF-2. $Pd(PPh_3)_4$ (0.098 g, 0.085 mmol) was dissolved in 10 mL of dry DMSO under N_2. **3** (0.31 g, 0.60 mmol), **4** (0.88 g, 1.0 mmol), and K_2CO_3(aq) (2 M, 4 mL; N_2 bubbled before use) were added to the solution. After the mixture was stirred at 100 ˚C for 48 h, the solvent was removed under vacuum. The resulting solid was dissolved in methanol and the solution was filtered by sellaite chromatography. The solvent was removed under vacuum, the resulting solid was suspended in acetone (100 mL) and the suspension was refluxed for 5h. The precipitated was collected by filtration, washed with chloroform (50 mL) and dried under vacuum to give **OF-2** as a yellowish brown powder (0.27 g, 48%). ^{1}H NMR (400 MHz, D_2O): 7.07-7.83 (22H), 6.03 (2H), 5.00 (2H), 3.12 (4H), 2.28 (8H), 2.00 (4H), 1.39-1.44 (8H), 0.67 (4H).

1.2.5. Synthesis of 2-ethynyl-9,9-bis(6-*N*,*N*,*N*-trimethylammoniumhexyl)fluorene (5).



8 **9** **5**

$PdCl_2(PPh_3)_2$ (0.070 g, 0.10 mmol), CuI (0.019 g, 0.10 mmol), and 2-bromo-9,9-bis(6-bromohexyl)fluorene (1.1 g, 1.8 mmol) was dissolved in 25 mL of dry diisopropylamine. Trimethylsilyl acetylene (0.34 mL, 2.4 mmol) was added to the solution. After the reaction solution was stirred at 70 ˚C for 8 h, the solvent was removed under vacuum. The resulting paste was purified by silica gel column chromatography (eluent = chloroform/hexane = 1/1 (*v*/*v*)). The solvents were removed under vacuum and the resulting paste was dried under vacuum to give **8** as a brown paste (0.95 g, 88%). ^1H NMR (500 MHz, $CDCl_3$): 7.67 (d, *J* = 4.0 Hz, 1H), 7.61 (d, *J* = 6.4 Hz, 1H), 7.43 (dd, *J* = 1.2 and 8.0 Hz, 1H), 7.41 (s, 1H), 7.29-7.31 (m, 3H), 3.26 (t, *J* = 5.2 Hz, 4H), 1.94 (t, *J* = 6.4 Hz, 4H), 1.63 (m, 4H), 1.94 (m, 4H), 1.62 (m, 4H), 0.55 (m, 4H), 0.27 (s, 9H).

A THF solution (10 mL) of **8** (0.95 g, 1.8 mmol) was added to an aqueous solution of KOH (20wt%, 1 mL). After the reaction solution was stirred at 20 ˚C for 1 h, the solvents were removed under vacuum. The resulting paste was dissolved in chloroform (100 mL) and washed with water (100 mL). The organic solution was dried over sodium sulfate and then the solvent was removed under vacuum. After the resulting paste was purified by silica gel column chromatography (eluent = chloroform/hexane = 1/1 (*v*/*v*)), the solvents were removed under vacuum and the resulting solid was dried under vacuum to give **9** as a brown paste (0.77 g, 85%). ^1H NMR (400 MHz, $CDCl_3$): 7.64 (d, *J* = 4.0 Hz, 1H), 7.60 (d, *J* = 6.0 Hz, 1H), 7.44 (d, *J* = 1.2 Hz, 1H), 7.43 (d, *J* = 1.2 Hz, 1H), 7.21-7.31 (m, 3H), 3.23 (t, *J* = 5.2 Hz, 4H), 3.10 (s, 1H), 1.91 (t, *J* = 6.4 Hz, 4H), 1.61 (t, *J* = 5.2 Hz, 4H), 1.11

(m, 4H), 1.04 (m, 4H), 0.53 (m, 4H).

9 (0.77 g, 1.5 mmol) was dissolved in dry THF (1 mL). After a THF solution of triethylamine (2M, 9.0 mL) was added to the solution, the reaction solution was stirred at 20 ˚C for 24h. The precipitate was collected by filtration, washed with hexane (50 mL) and dried under vacuum to give **5** as a white powder (0.85 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.71-7.75 (2H), 7.58 (1H), 7.45-7.48 (2H), 7.36 (2H), 3.55 (1H), 2.97 (4H), 2.90 (18H), 1.96 (4H), 1.34 (4H), 0.94 (8H), 0.41 (4H).

1.2.6. Synthesis of OF-3. $Pd(PPh_3)_4$ (0.052 g, 0.050 mmol) was dissolved in 15 mL of dry DMF under N_2. **2** (0.22 g, 0.20 mmol), **5** (0.29 g, 0.45 mmol), CuI (0.010 g, 0.053 mmol), and dry Et_3N (8 mL) were added to the solution. After the reaction solution was stirred at 55 ˚C for 24 h, the solvent and BOC were removed under vacuum at 50 ˚C and then the residue was extracted with water (100 mL) and washed with chloroform (100 mL). The solvent was removed under vacuum. The resulting solid was washed with acetone (50 mL) twice, collected by filtration and dried under vacuum to yield **OF-3** as an orange powder (0.22 g, 61%). ^{1}H NMR (400 MHz, DMSO-d_6): 7.37-7.96 (22H), 5.62 (2H), 3.51 (4H), 3.15 (8H), 2.97 (36H), 2.09 (8H), 1.44 (8H), 1.02-1.09 (16H), 0.50 (12H).

2. Supplementary Figures

2.1. IR spectra.

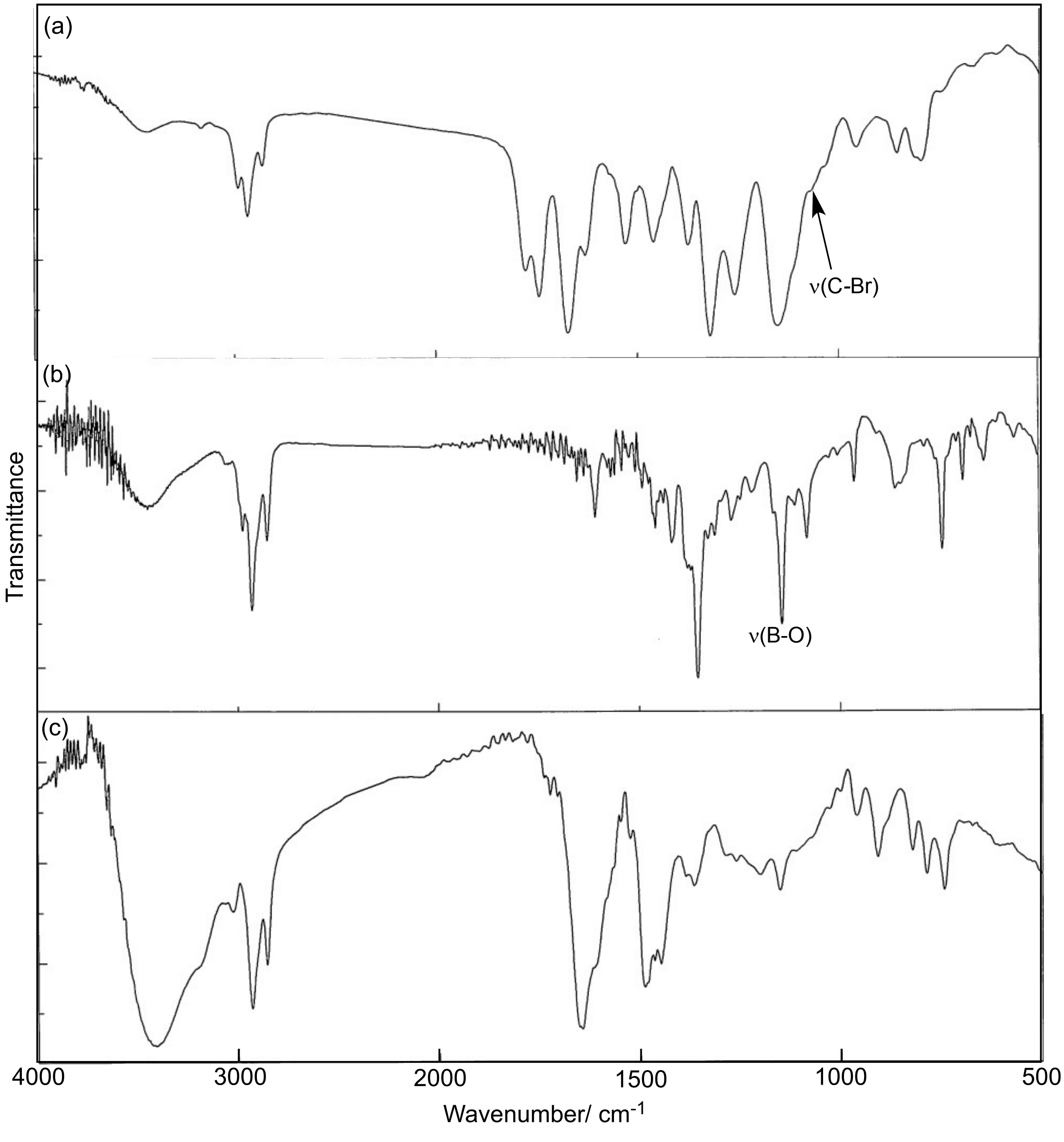


Figure S1a. IR spectra of (a) **2**, (b) **1** and (c) **OF-1**.

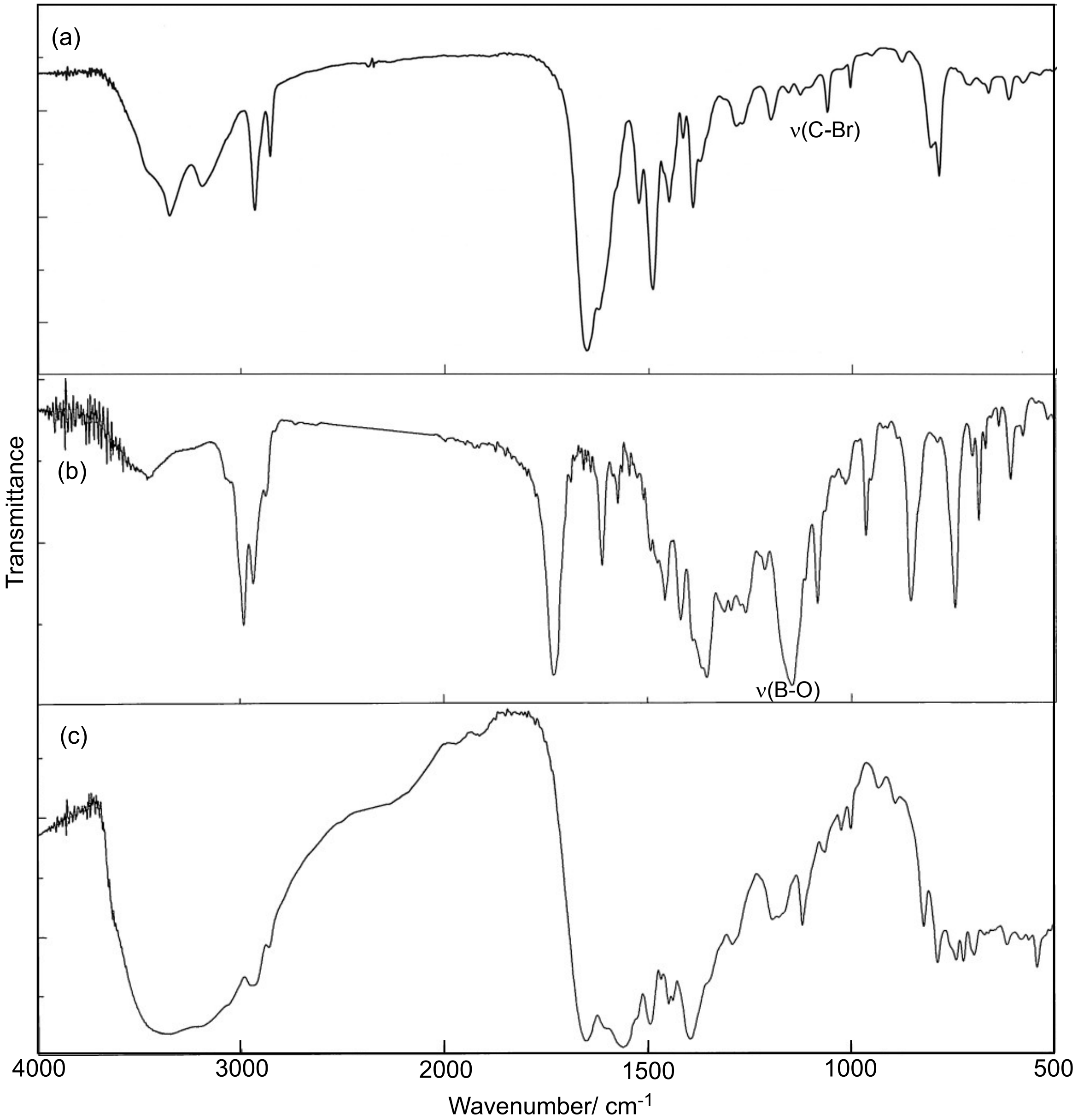


Figure S1b. IR spectra of (a) **2**, (b) **3** and (c) **OF-2**.

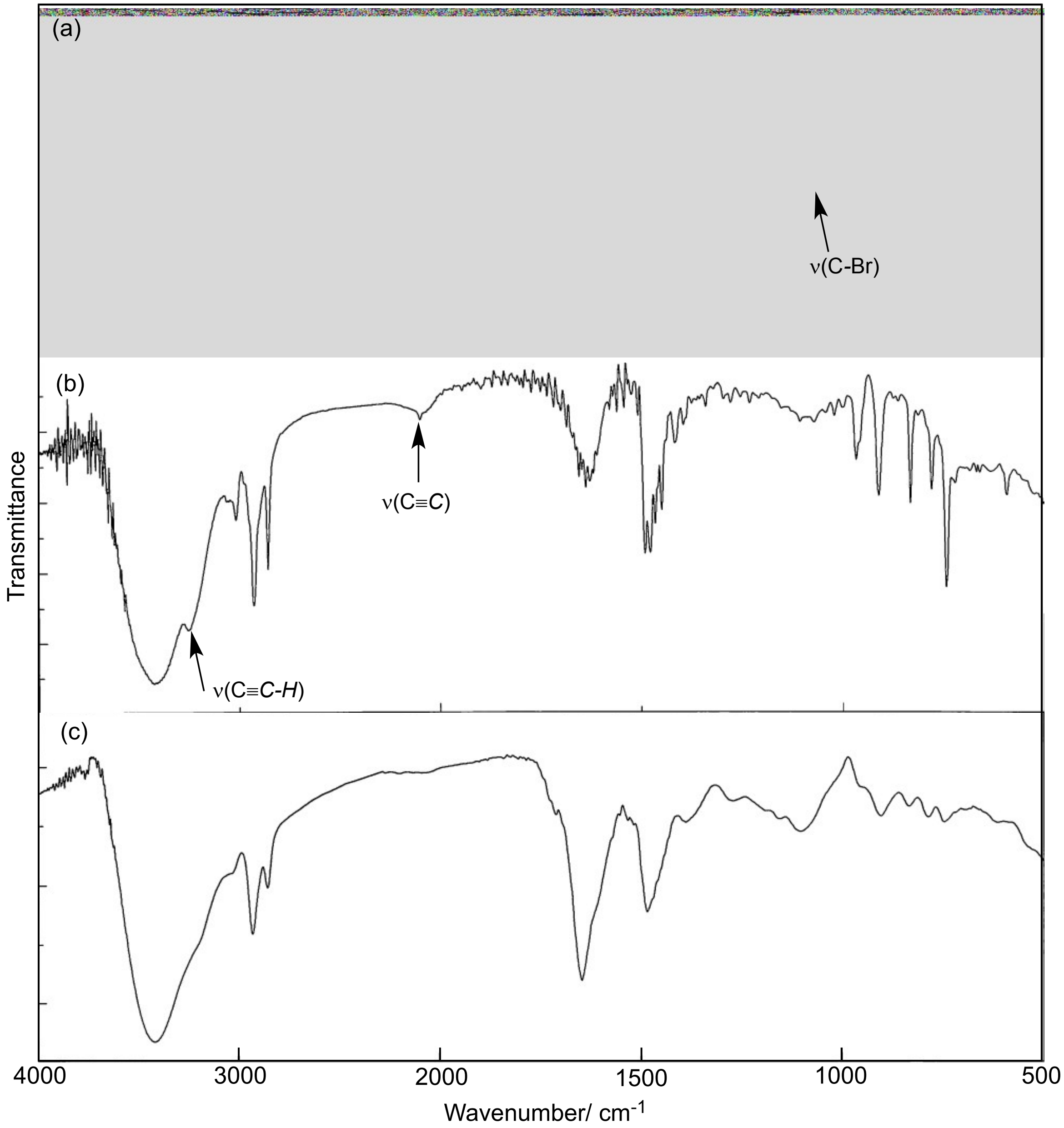


Figure S1c. IR spectra of (a) **2**, (b) **5** and (c) **OF-3**.

2.2. ^{1}H NMR spectra

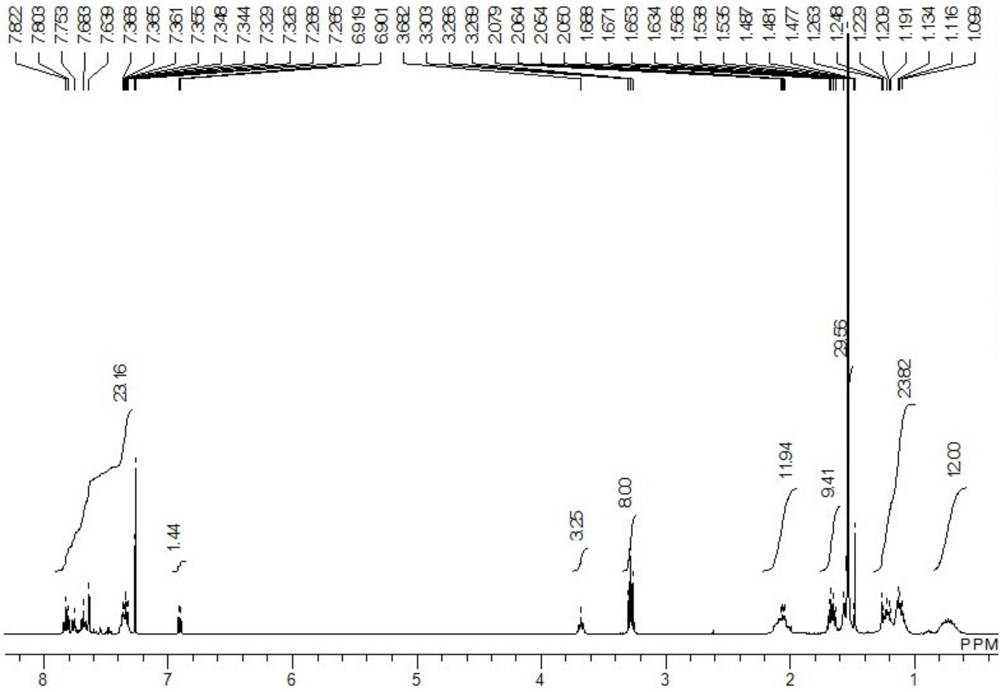


Figure S2a. ^{1}H NMR spectrum of **6** in $CDCl_3$.

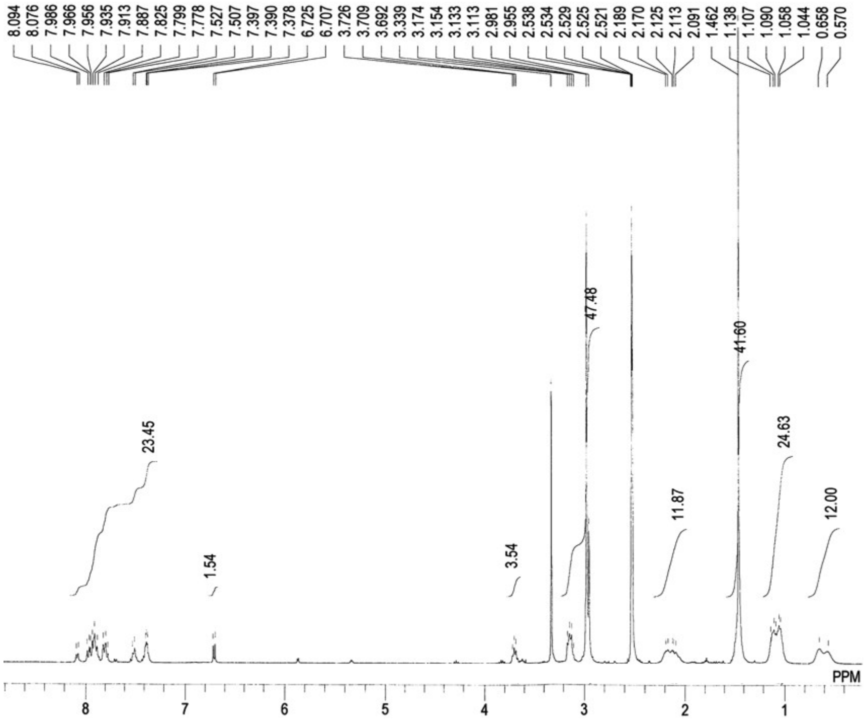


Figure S2b. ^{1}H NMR spectrum of **7** in DMSO-d_6.

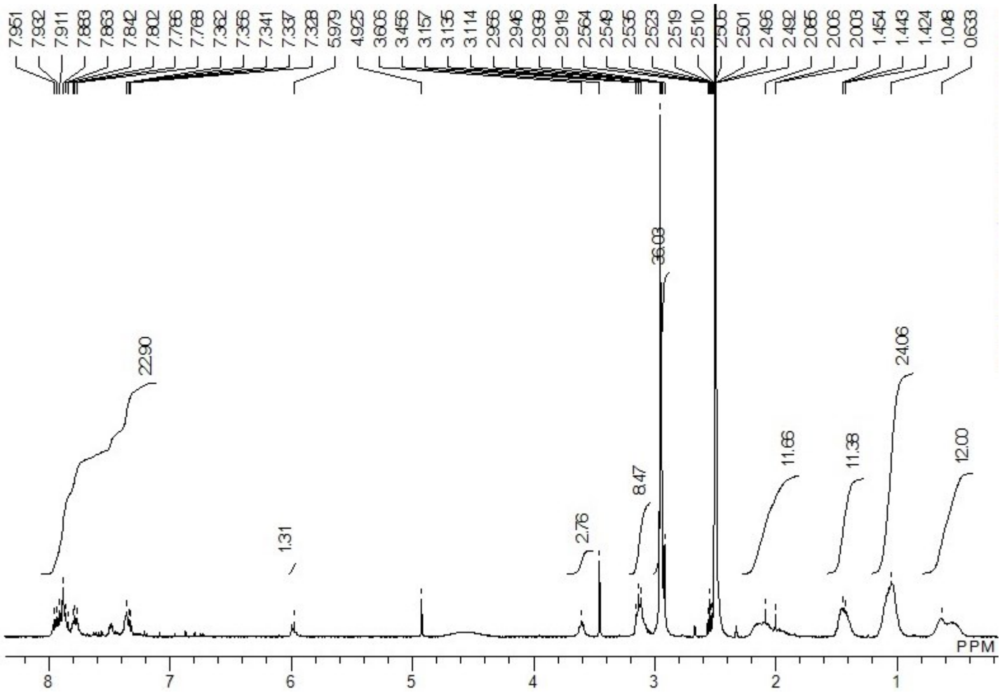


Figure S2c. ^{1}H NMR spectrum of **OF-1** in DMSO-d_6.

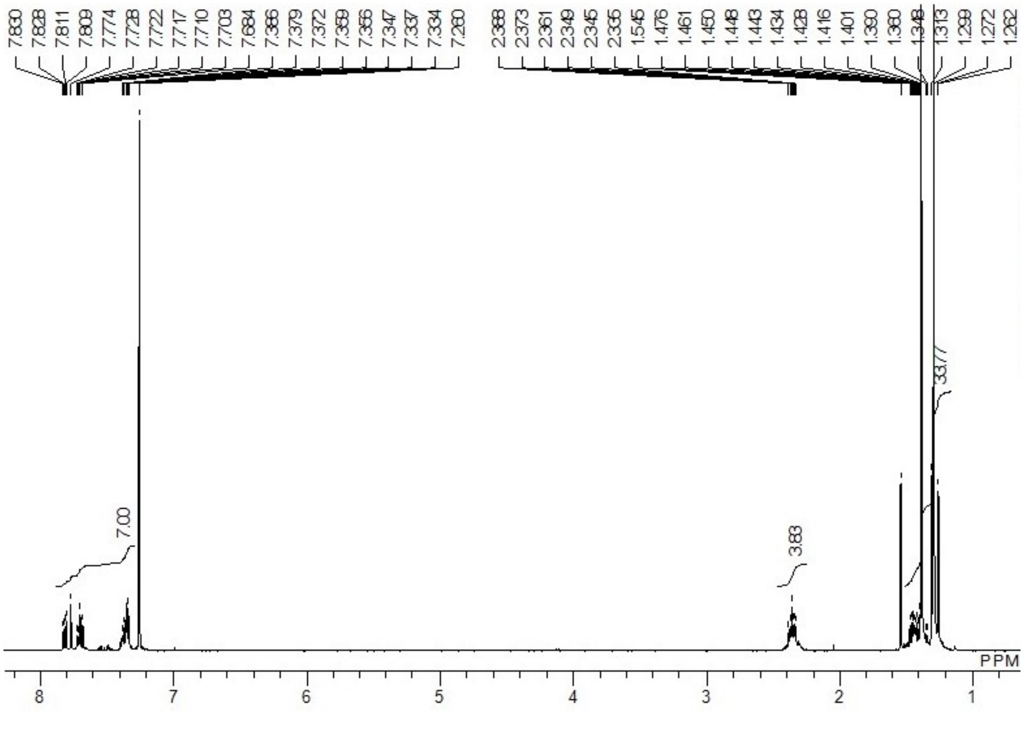


Figure S2d. ^{1}H NMR spectrum of **3** in CDCl$_3$.

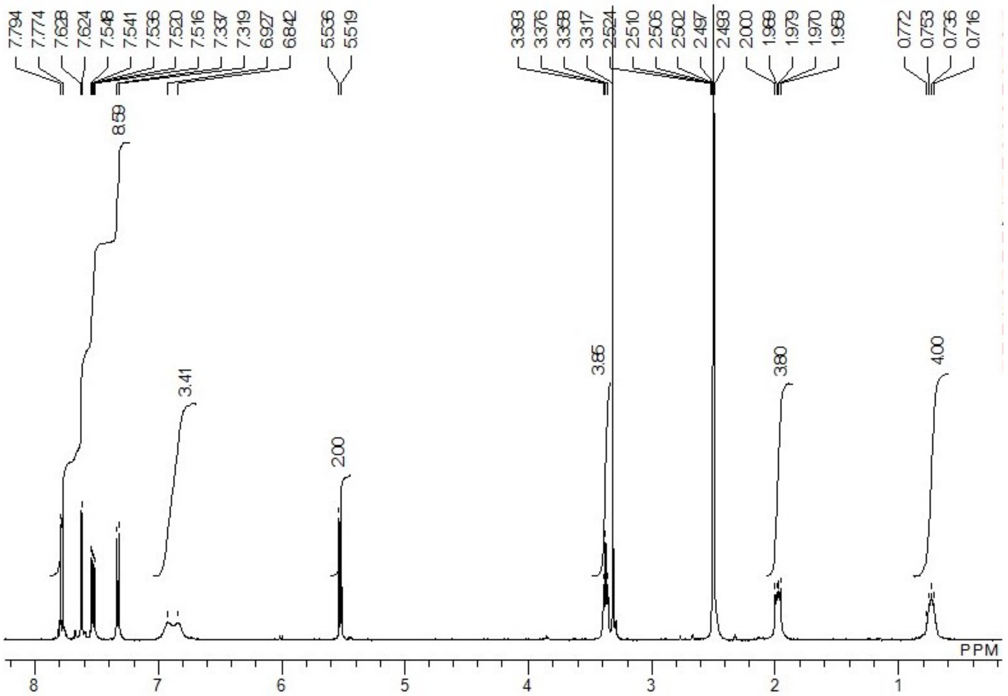


Figure S2e. ^{1}H NMR spectrum of **4** in DMSO-d_6.

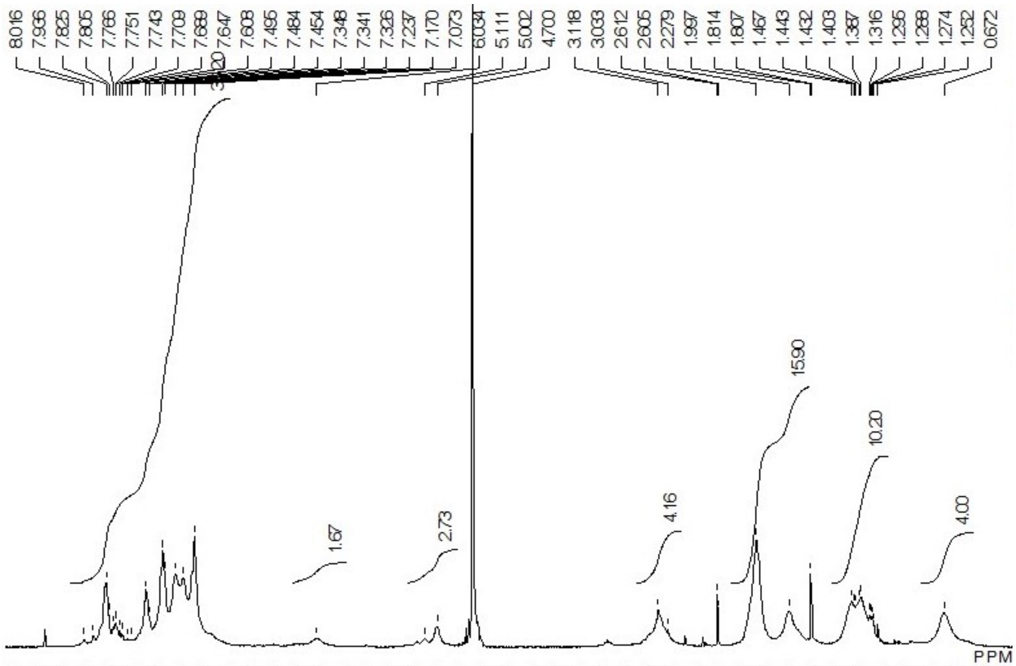


Figure S2f. ^{1}H NMR spectrum of **OF-2** in D_2O.

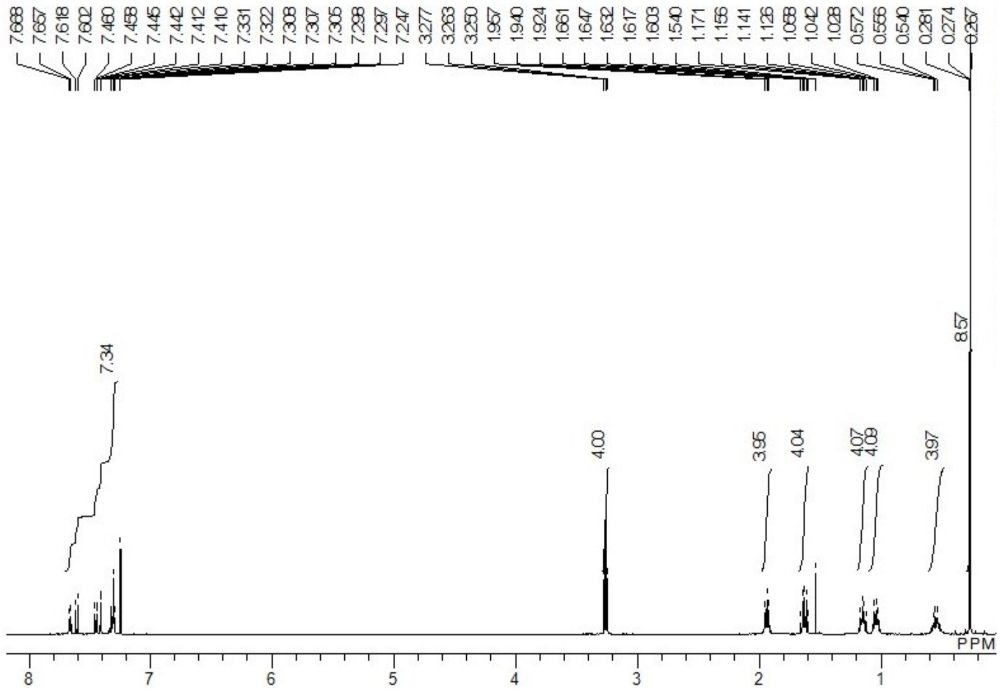


Figure S2g. ^{1}H NMR spectrum of **8** in CDCl$_3$.

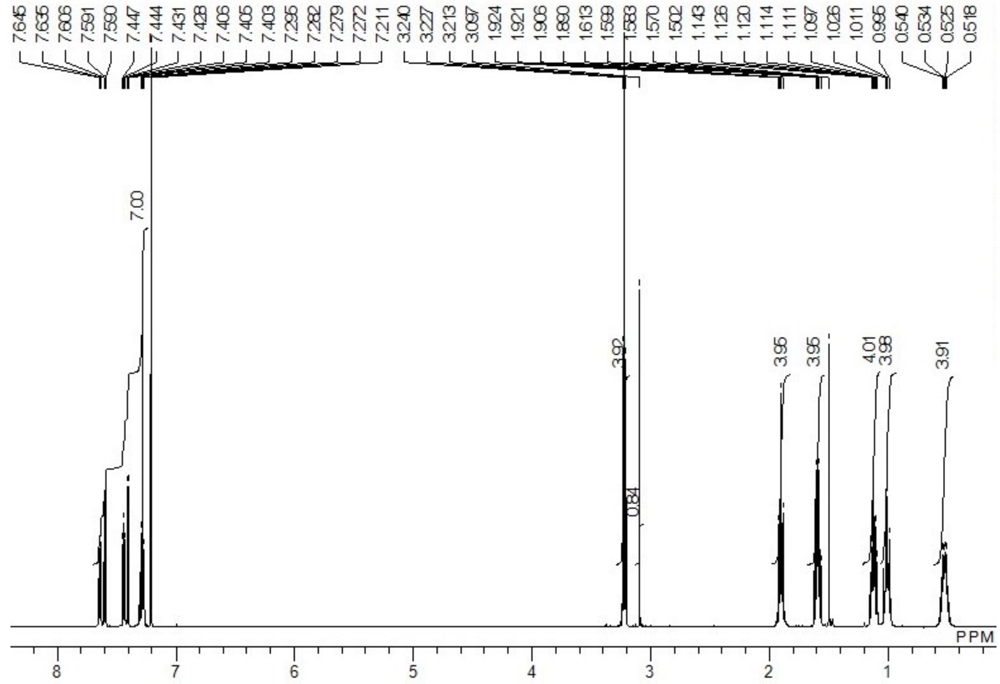


Figure S2h. ^{1}H NMR spectrum of **9** in CDCl$_3$.

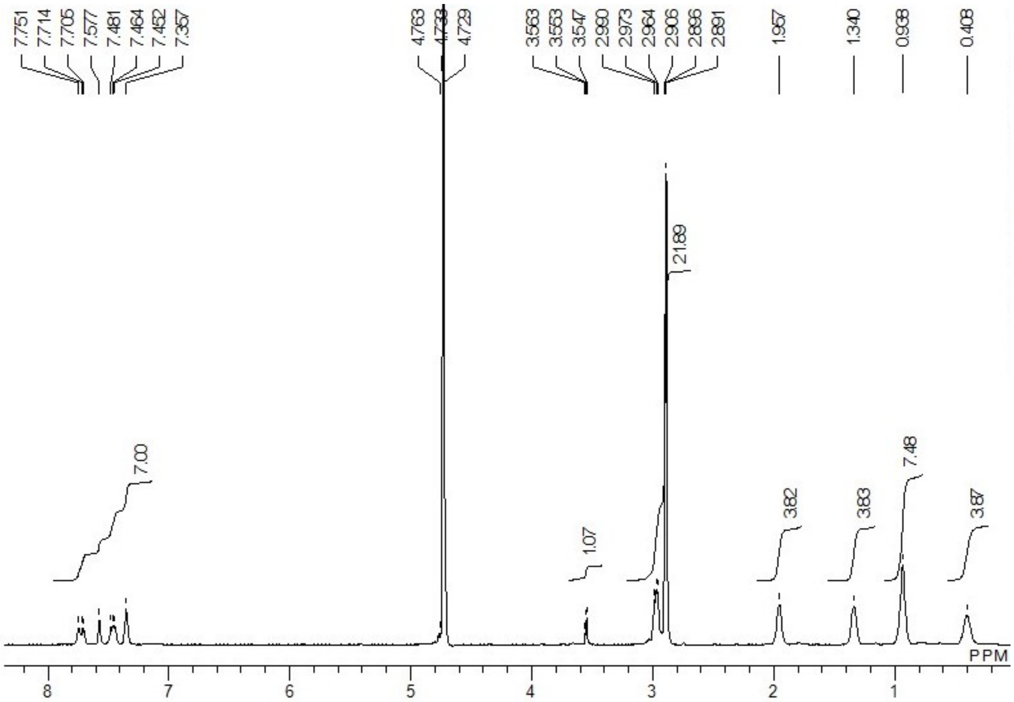


Figure S2i. ^{1}H NMR spectrum of **5** in D_2O.

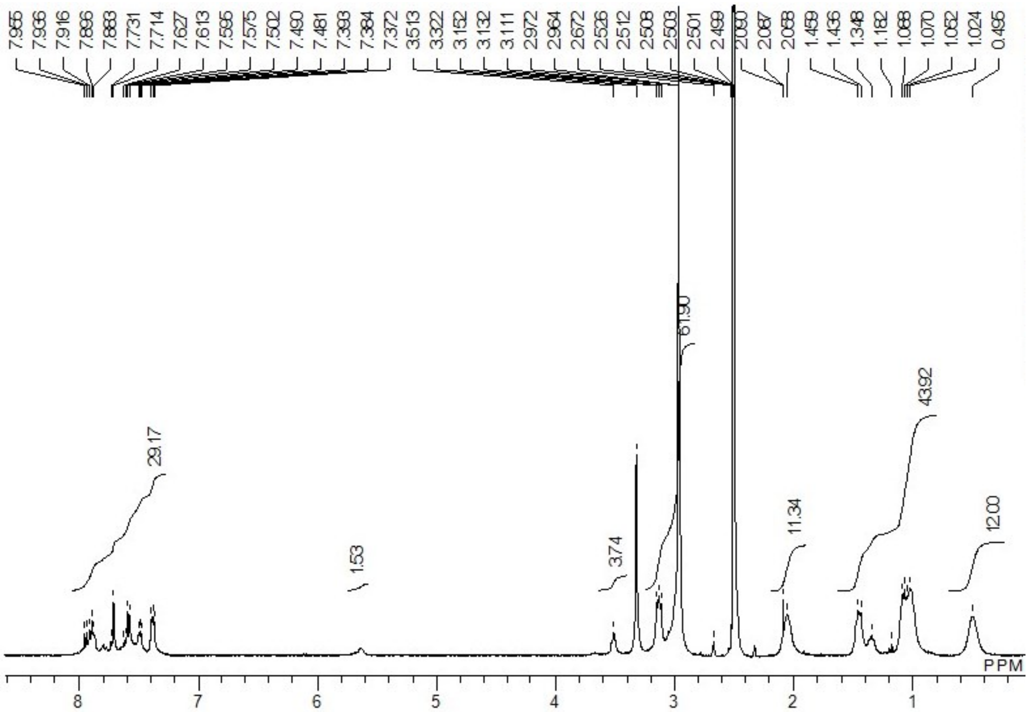


Figure S2j. ^{1}H NMR spectrum of **OF-3** in DMSO-d_6.

2.3. Cyclic voltammograms

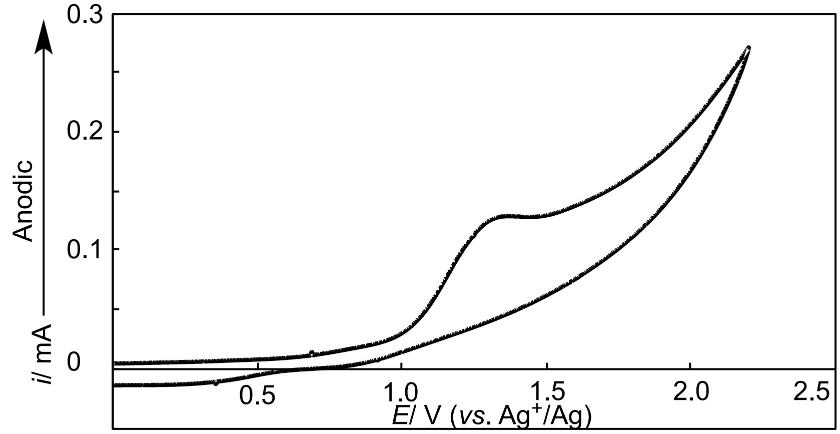


Figure S3a. Cyclic voltammogram of the cast film of **OF-2** on a Pt plate in an acetonitrile solution of $[Et_4N]BF_4$.

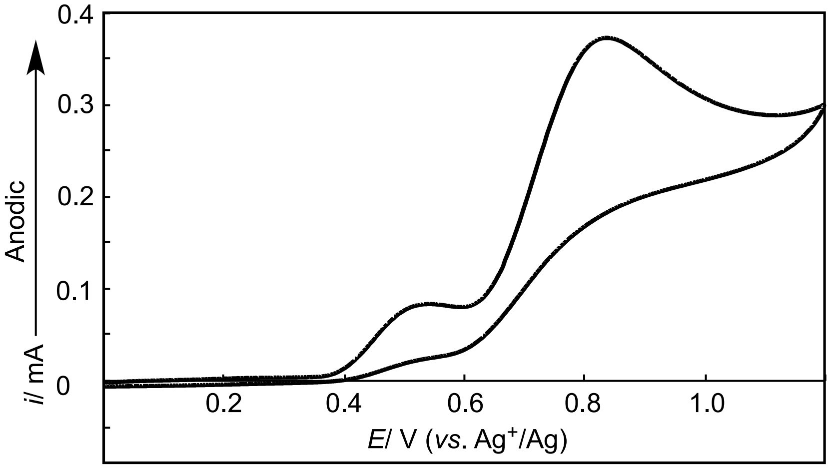


Figure S3b. Cyclic voltammogram of **OF-3** in a DMSO solution of $[Et_4N]BF_4$.

2.4. UV-vis and PL spectra and fluorescence decay profiles

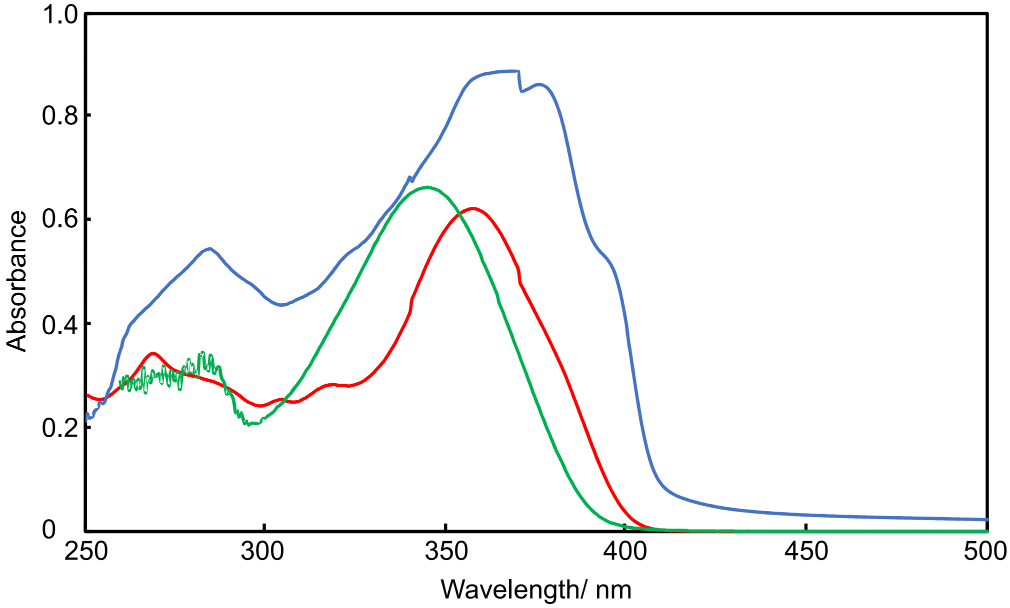
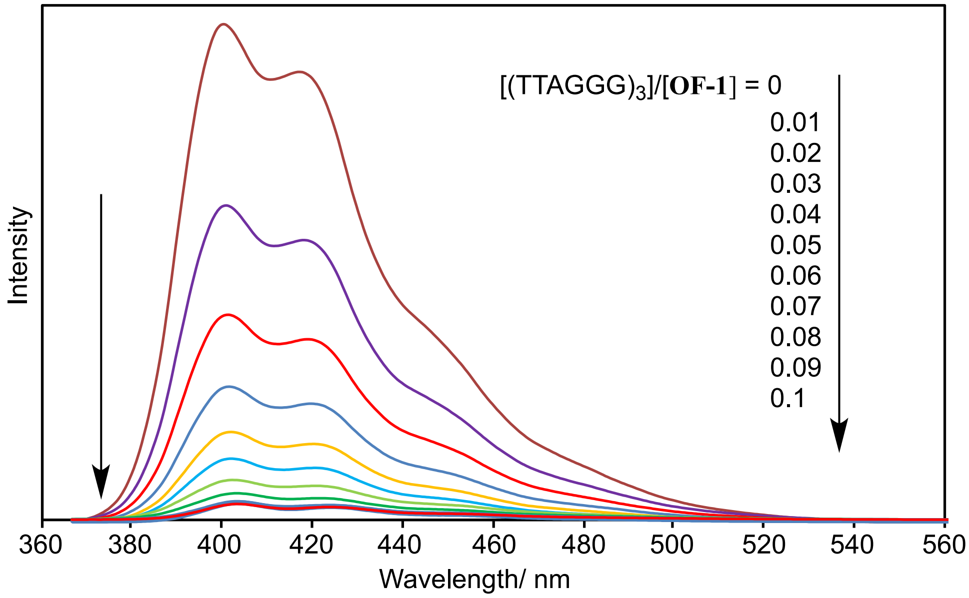
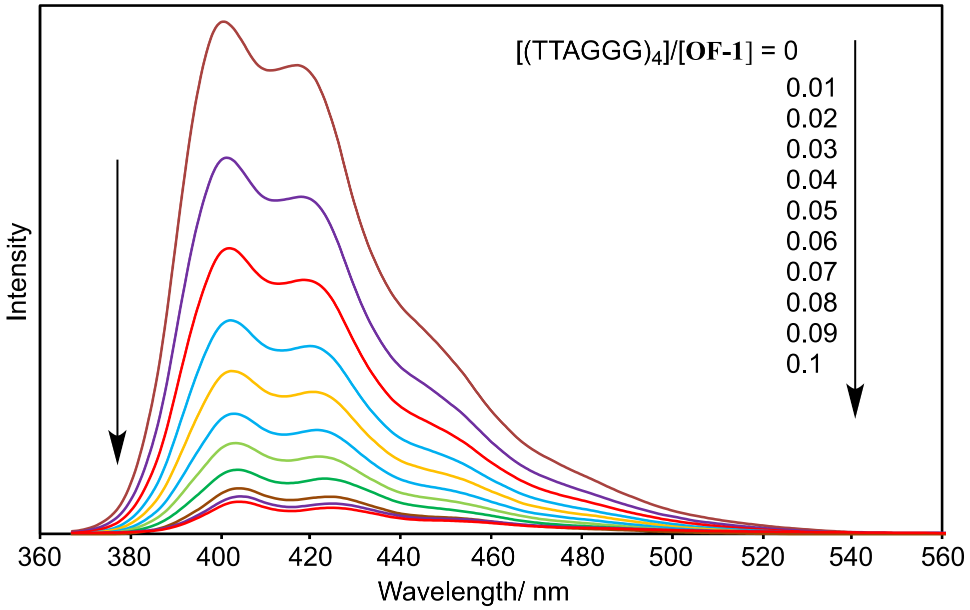


Figure S4. UV-vis spectra of the aqueous solutions of **OF-1** (red curve), **OF-2** (green curve) and **OF-3** (blue curve).





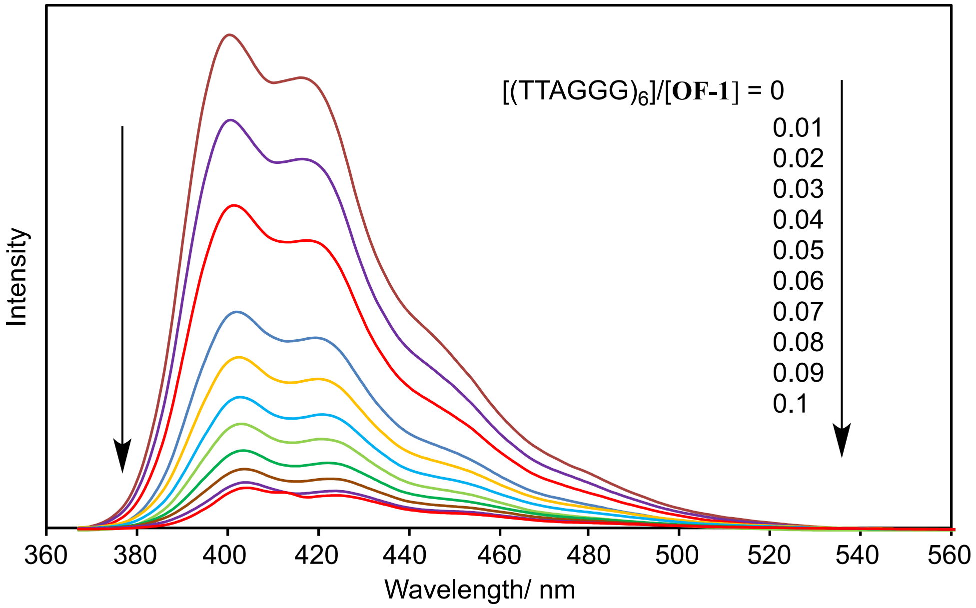


Figure S5. PL spectra of the aqueous solutions of **OF-1** in the presence of various amounts of (TTAGGG)$_m$.

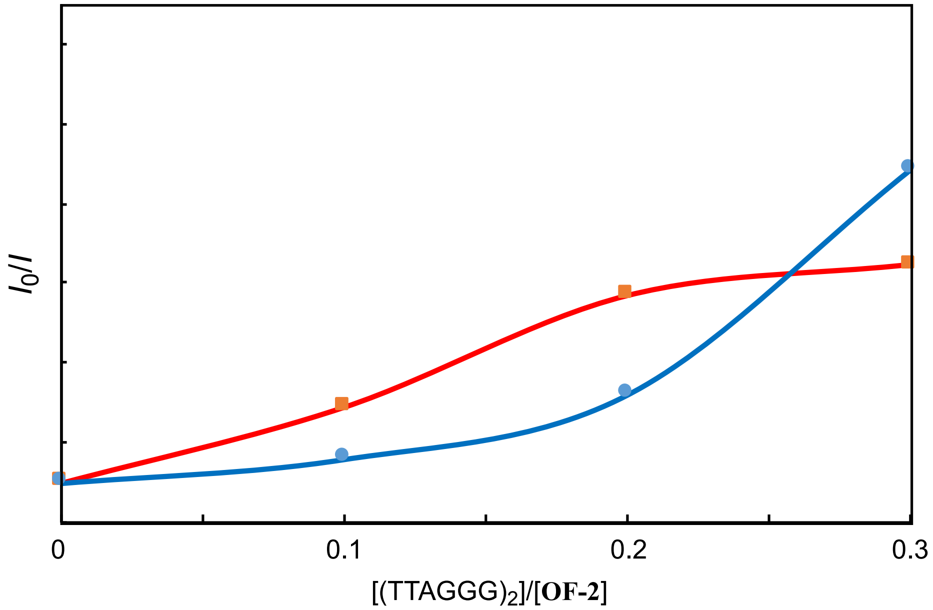


Figure S6. (TTAGGG)$_2$ concentration dependence of I_0/I values of **OF-1** in the absence (blue plots) and presence of NaCl (red plots).

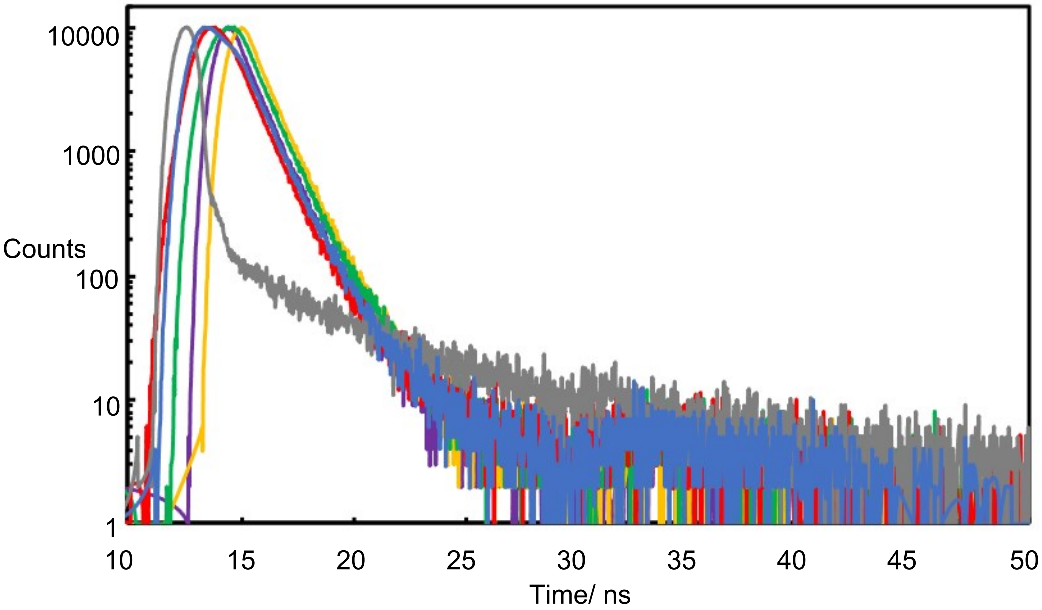
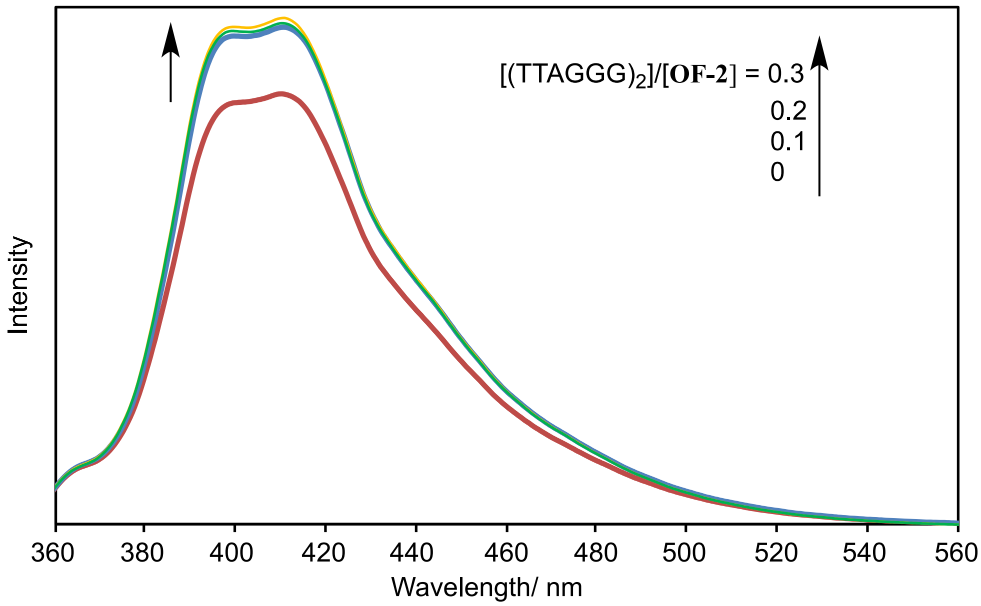
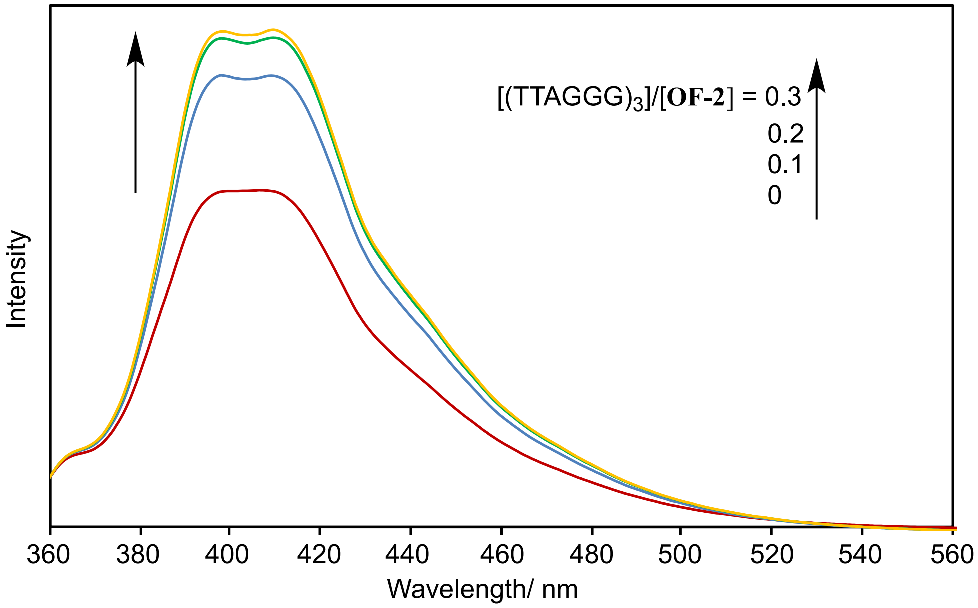
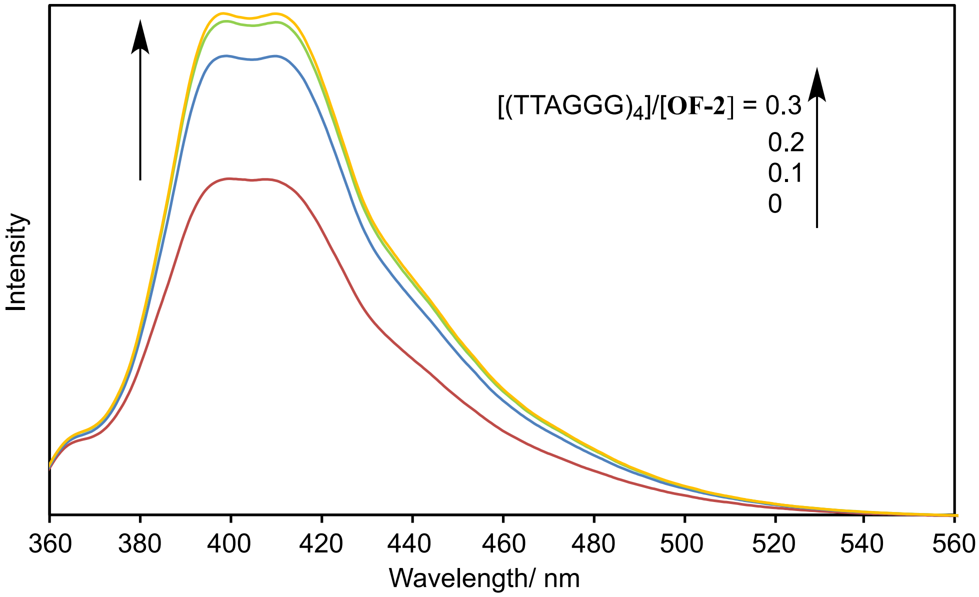


Figure S7. Fluorescence decay profiles of the aqueous solutions of **OF-1** before (blue curve) and after addition of 0.06 equimolar amount of (TTAGGG)$_m$ (red curve for m = 2, green curve for m = 3, yellow curve for m = 4 and purple curve for m = 6).


[(TTAGGG)$_2$]/[**OF-2**] = 0.3
0.2
0.1
0
Intensity
360
380
400
420
440
460
480
500
520
540
560
Wavelength/ nm


[(TTAGGG)$_3$]/[**OF-2**] = 0.3
0.2
0.1
0
Intensity
360
380
400
420
440
460
480
500
520
540
560
Wavelength/ nm



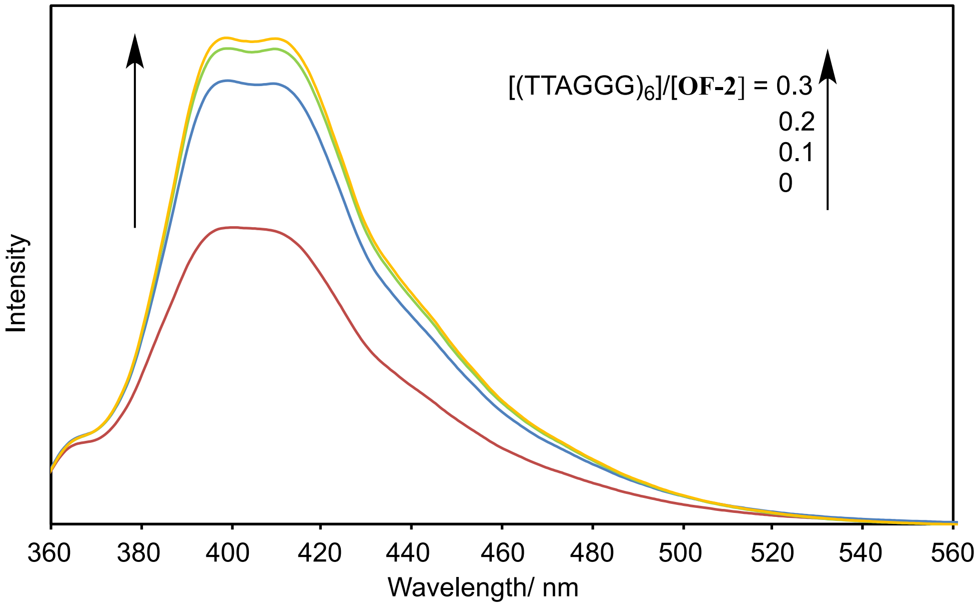


Figure S8. PL spectra of the aqueous solutions of **OF-2** in the presence of various amounts of (TTAGGG)$_m$.

2.5. ^{1}H NMR spectra

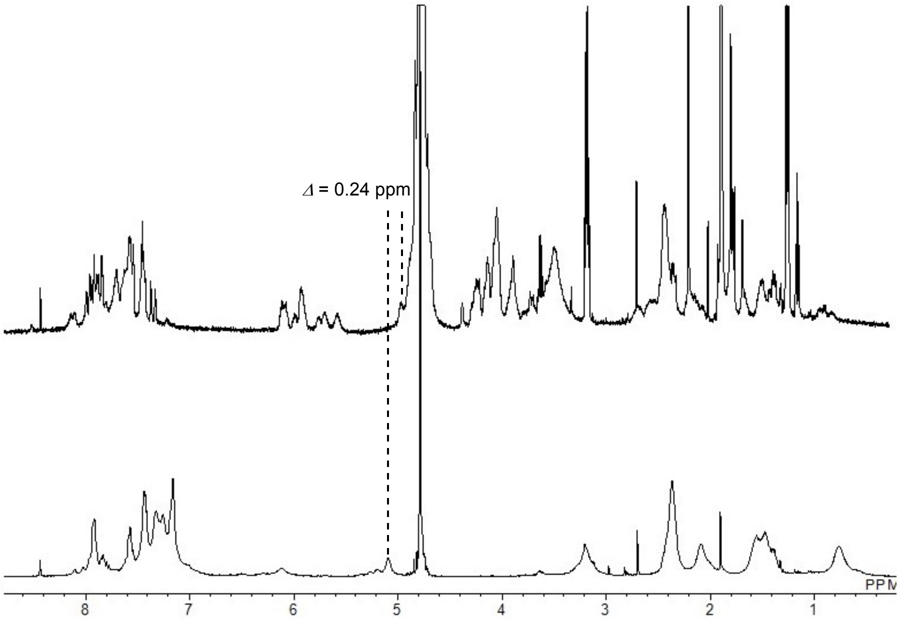


Figure S9. ^{1}H NMR spectra of **OF-2** in the absence (upper) and presence of 0.06 equimolar amount of $(TTAGGG)_2$ (lower) in D_2O.

2.6. Electrical conductivity

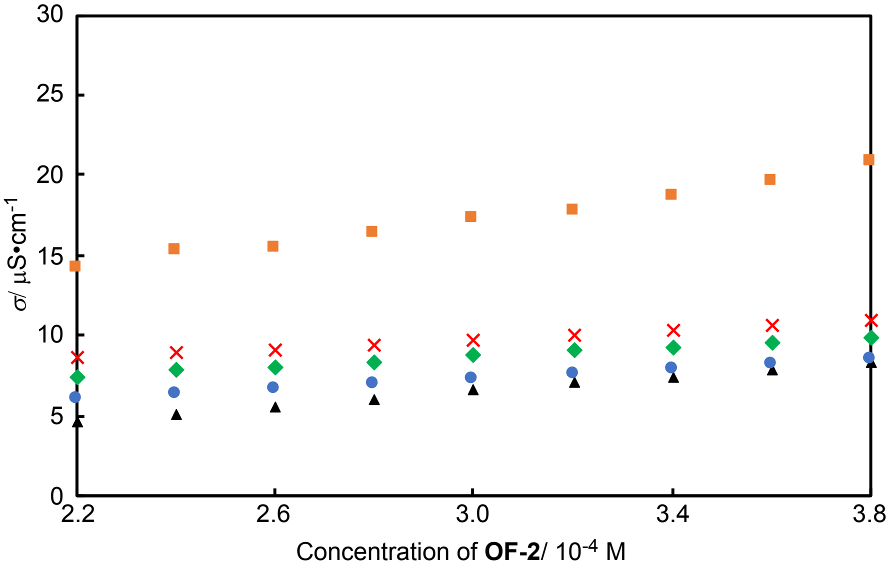


Figure S10. Electrical conductivity of the aqueous solutions of **OF-2** in the absence (black plots) and presence of 2.2×10^{-4} M of $(TTAGGG)_m$ (blue plots for $m = 2$, green plots for $m = 3$, red plots for $m = 4$ and orange plots for $m = 6$).

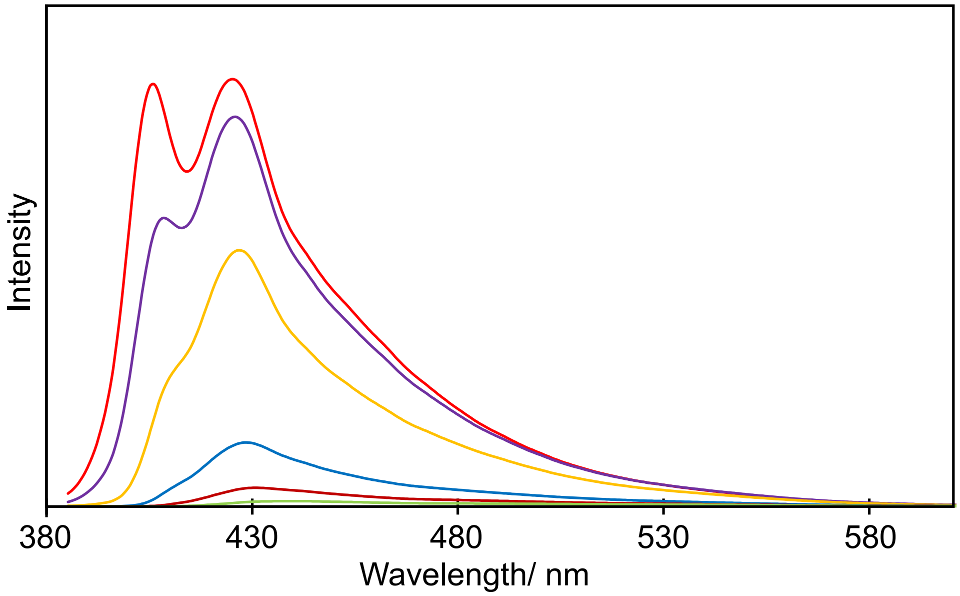


Figure S11. PL spectra of the aqueous solutions of **OF-3** in various concentrations ($c = 1 \times 10^{-5}$ M; red curve, $c = 0.5 \times 10^{-4}$ M; purple curve, $c = 1 \times 10^{-4}$ M; yellow curve, $c = 0.5 \times 10^{-3}$ M; blue curve, $c = 0.5 \times 10^{-3}$ M; dark red curve, and $c = 1 \times 10^{-3}$ M; green curve.

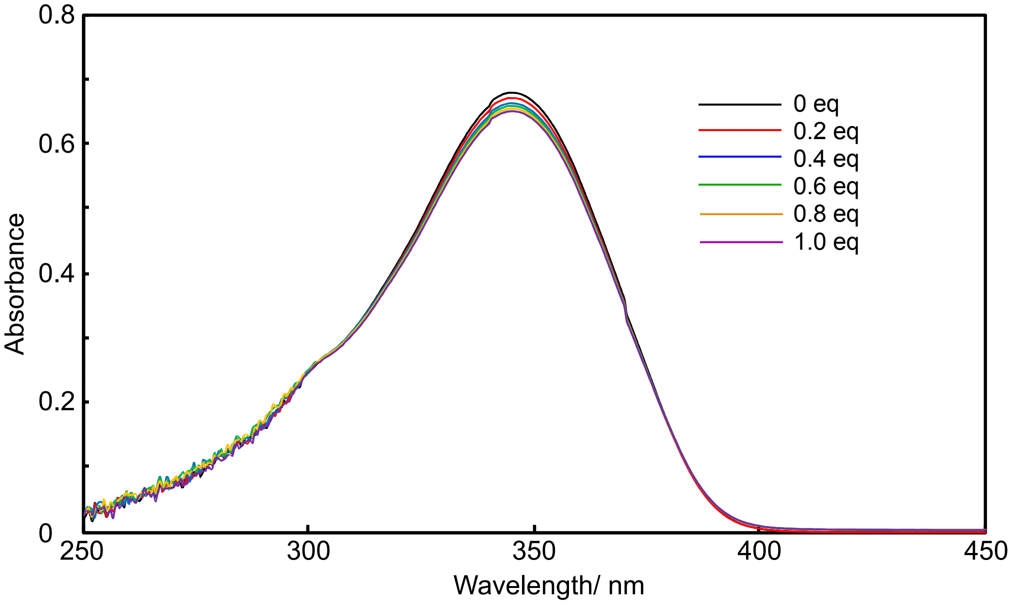


Figure S12. UV-vis spectra of the aqueous solution of **OF-2** in the presence of various amounts of $(TTAGGG)_2$.

3. Supporting References

1. R. Yang, H. Wu, Y. Cao and, G. C. Bazan, *J. Am. Chem. Soc*. 2006, **128**, 14422.

2. I. Yamaguchi and K. Miyawaki, *React. Funct. Polym*. 2017, **120**, 14.

3. S.-H. Oh, S.-I. Na, Y.-C. Nah, D. Vak, S.-S. Kim and S.-Y. Kim, *Org. Electron*. 2007, **8**, 773.

4. Y. Zhang, B. Liu and Y. Cao, *Chem. Asian J*. 2008, **3**, 739.

5. X. Liu, R. Zhu, Y. Zhang, B. Liu and S. Ramakrishna, *Chem. Commun*. 2008, **44**, 3789.