# CHAPTER II

# EXPERIMENTAL

## 2.1 Chemicals and materials

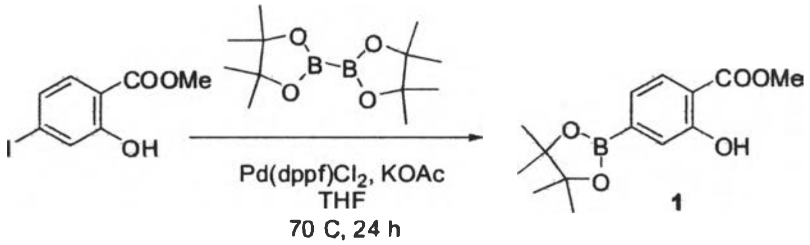
All reagents were purchased from Sigma-Aldrich. Metal ions were prepared from their commercially available inorganic salts purchased from Sigma-Aldrich. For most reactions, solvents such as methylene chloride ($CH_2Cl_2$) and methanol (MeOH) were reagent grade stored over molecular sieves. In anhydrous reactions, solvents such as THF and TEA were dried before use according to the standard procedures. Solvents used for extraction and chromatography such as $CH_2Cl_2$, hexane, ethyl acetate (EtOAc) and MeOH were commercial grade. Column chromatography was operated using Merck silica gel 60 (70-230 mesh). Thin layer chromatography (TLC) was performed on silica gel plates (Merck $F_{245}$). The most reactions were carried out under positive pressure of $N_2$ filled in rubber balloons.

## 2.2 Analytical instruments

Mass spectra were recorded on a Microflex MALDI-TOF mass spectrometer (Bruker Daltonics) using dithanol as a matrix. $^1H$- and $^{13}C$-NMR spectra were acquired from sample solution in $CDCl_3$ and DMSO-*d6* on Varian Mercury NMR spectrometer (Varian, USA) at 400 MHz and 100 MHz, respectively. The UV-visible absorption spectra were obtained from a Varian Cary 50 UV-Vis spectrophotometer (Varian, USA) and the fluorescence emission spectra were recorded on a Varian Cary Eclipse spectrofluorometer (Varian, USA).

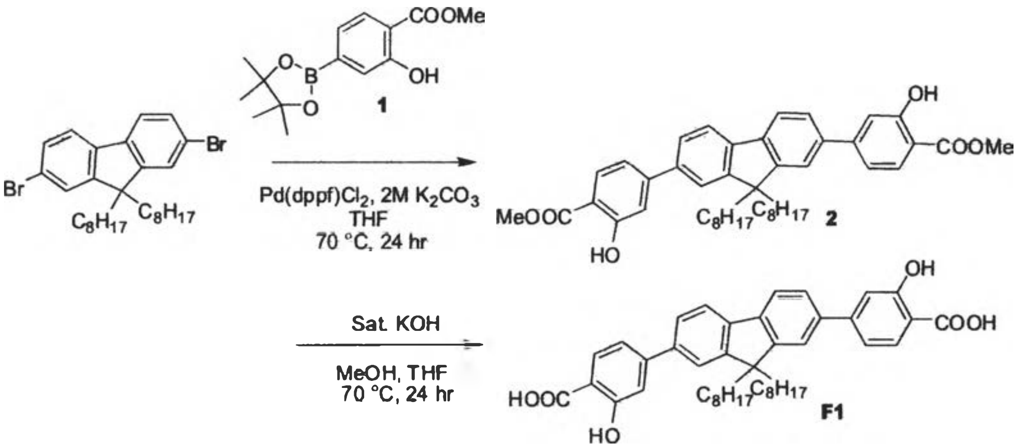
## 2.3 Synthesis

### 2.3.1 Preparation of methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)salicylate (**1**)



A mixture of methyl 4-iodosalicylate (0.50 g, 1.8 mmol), bis(pinacolato)diboron (0.69 g, 2.7 mmol), Pd(dppf)Cl$_2$ (66 mg, 0.09 mmol) and KOAc (0.71 g, 7.2 mmol) was dissolved in THF (5 mL). The reaction mixture was heated up to 70 °C for 18 h. The mixture was extracted with $CH_2Cl_2$ (2 x 20 mL). The combined organic phase was washed with water (2 x 25 mL) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the residue was eluted through a silica gel column by solvents hexane/$CH_2Cl_2$ (4/1 v/v) as an eluent to afford methyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)salicyate as a white solid. (0.311 g, 62% yield). $^1H$ NMR (400 MHz, $CDCl_3$) δ 10.61 (s, 1H), 7.81 (d, *J* = 7.9 Hz, 1H), 7.41 (s, 1H), 7.27 (d, *J* = 7.9 Hz, 1H), 3.96 (d, *J* = 12.0 Hz, 3H), 1.35 (d, *J* = 10.1 Hz, 12H). The data were agreed with those reported in literature [14].

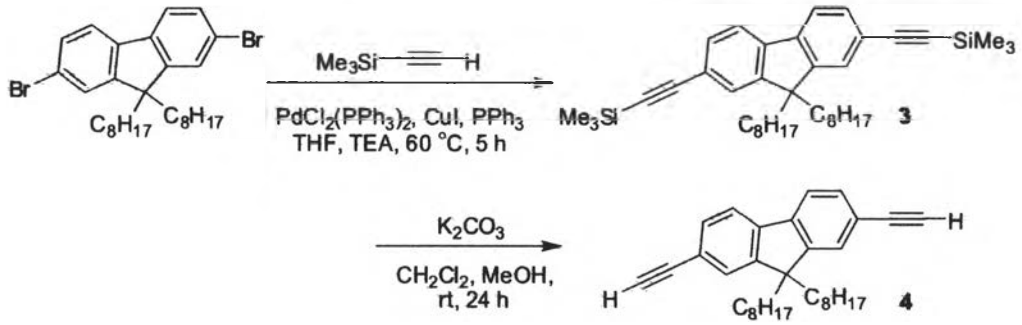
### 2.3.2 Preparation of **F1**



A mixture of **1** (0.21 g, 0.76 mmol), 2,7-dibromo-9,9-dioctyl-9H-fluorene ( 0.20 g, 0.34 mmol), Pd(dppf)Cl$_2$ (13 mg, 0.02 mmol) dissolved in THF (5 mL). A solution of

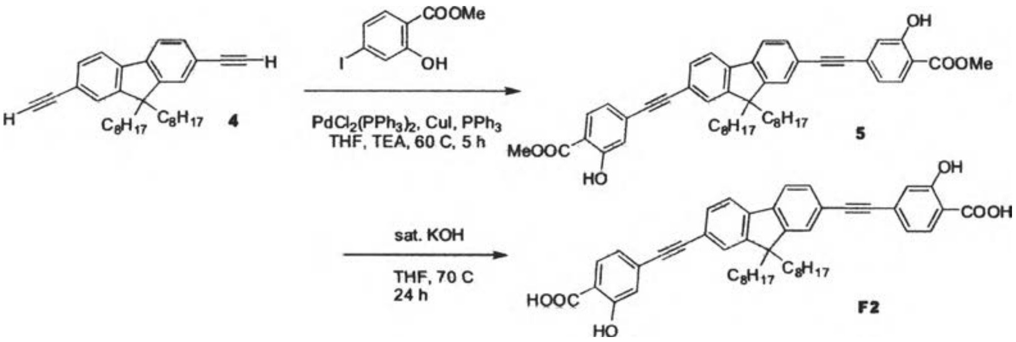
$K_2CO_3$ (2.0 M) was added and mixture was heated up to 70 °C for 24 h. The mixture was extracted with $CH_2Cl_2$ (2 x 20 mL). The combined organic phase was washed with water (2 x 25 mL) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the crude product was purified by a silica column chromatography using hexane : EtOAc (20:1) as an eluent to afford **2** as a white solid. $^1H$ NMR (400 MHz, $CDCl_3$) δ 10.85 (s, 1H), 7.92 (d, J = 8.3 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H), 7.59 (s, 1H), 7.30 (s, 1H), 7.21 (d, J = 8.3 Hz, 1H), 3.99 (s, 3H), 2.03 (d, J = 12.3 Hz, 2H), 1.29 – 0.96 (m, 12H), 0.78 (t, J = 7.0 Hz, 3H). A mixture of **2** (0.15 g, 0.22 mmol) in THF (2 mL) and MeOH (2 mL) was added with saturated KOH aqueous solution (0.1 mL) and the mixture was heated to 70 °C. After 24 h the solution was evaporated and the residue was dissolved in water (20 mL). The aqueous layer was acidified by addition of 6N HCl and centrifuged. The precipitates were washed with water until neutral to afford **F1** as a yellow solid in 50% overall yield. $^1H$ NMR (400 MHz, DMSO-*d6*) δ 7.93 (d, *J* = 7.9 Hz, 1H), 7.87 (d, *J* = 8.7 Hz, 1H), 7.83 (s, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.34 (s, 1H), 7.33 (d, *J* = 4.2 Hz, 1H), 2.10 (d, *J* = 8.1 Hz, 2H), 1.25 – 0.92 (m, 12H), 0.71 (t, *J* = 6.9 Hz, 3H). $^{13}C$ NMR (100 MHz, DMSO-*d6*): δ (ppm) 172.8, 161.5, 151.6, 147.4, 140.5, 137.8, 130.8, 126.0, 121.4, 120.6, 117.8, 114.7, 111.7, 55.2, 39.5, 31.0, 29.0, 28.34, 28.29, 23.2, 21.9, 13.78. MALDI-TOF *m/z* calcd for $C_{43}H_{50}O_6$, 662.361; found, 661.596.

### 2.3.3 Preparation of 2,7-diethynyl-9,9-dioctyl-9H-fluorene (**4**)



A mixture of 2,7-dibromo-9,9-dioctyl-9H-fluorene (1.00 g, 1.8 mmol), $PdCl_2(PPh_3)_2$ (0.26 g, 0.36 mmol), CuI (0.13 g, 0.73 mmol), $PPh_3$ (0.19 g, 0.73 mmol) was dissolved in THF (10 mL) and TEA (20mL). Trimethylsilylacetylene (0.45 g, 4.6 mmol) was added and the mixture was stirred at 60 °C for 5 h. The reaction mixture was extracted with $CH_2Cl_2$ (2 x 20 mL). The combined organic phase was washed with water (2 x 25 mL) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the crude product was purified by a silica column chromatography using hexane as an eluent to afford **3** as a yellow oil. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.59 (d, J = 7.8 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.41 (s, 1H), 2.04 – 1.74 (m, 2H), 1.38 – 0.64 (m, 15H), 0.28 (s, 9H). A mixture of **3** (0.89 g, 1.53 mmol) and $K_2CO_3$ (43 mg, 0.31 mmol) in $CH_2Cl_2$ (10 mL) and MeOH (10 mL) was stirred at room temperature for 24 h. The reaction mixture was extracted with $CH_2Cl_2$ (2 x 50 mL). The organic phase was washed with water (20 x 2 mL) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the residue was eluted through a silica gel column chromatography using hexane/$CH_2Cl_2$ (4/1 v/v) as an eluent to afford **4** as a yellow oil in 77% overall yield. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.8 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.41 (s, 1H), 1.92 (d, *J* = 7.1 Hz, 1H), 1.29 – 0.94 (m, 12H), 0.82 (t, *J* = 7.1 Hz, 3H), 0.28 (s, 9H).

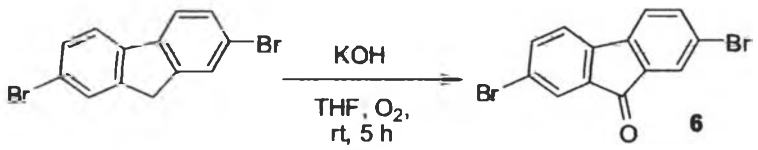
2.3.4 Preparation of **F2**



A mixture of **4** (0.20 g, 0.46 mmol), $PdCl_2(PPh_3)_2$ (64 mg, 0.09 mmol), CuI (35 mg, 0.18 mmol), $PPh_3$ (48 mg, 0.18 mmol) and methyl 4-iodosalicylate (0.28 g, 1.0 mmol) in THF (10 mL) and TEA (10 mL) was added and the mixture was stirred at 60 °C for 5 h. The reaction mixture was extracted with $CH_2Cl_2$ (2 × 20 mL). The combined organic phase was washed with water (2 × 25 mL) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the crude product was purified by a silica gel column chromatography using hexane as an eluent to yield **5** as a yellow oil. $^1H$ NMR (400 MHz, $CDCl_3$) δ 10.81 (s, 1H), 7.83 (d, *J* = 8.2 Hz, 1H), 7.68 (d, *J* = 7.3 Hz, 1H), 7.54 (d, *J* = 9.1 Hz, 1H), 7.53 (s, 1H), 7.18 (s, 1H), 7.07 (d, *J* = 9.1 Hz, 1H), 3.96 (s, 3H), 1.98 (d, 2H), 1.31 – 0.98 (m, 12H), 0.83 (t, *J* = 6.9 Hz, 3H). A mixture of **5** (0.20 g, 0.27 mmol) in THF (10 mL) was added with saturated KOH aqueous solution (0.3 mL) and the mixture was heated to 70 °C. After 24 h the solution was evaporated and the residue was dissolved in water (20 mL). The aqueous layer was acidified by addition of 6N HCl and centrifuged. The precipitates were washed by water until neutral to afford **F2** as a light yellow solid in 62% overall yield. $^1H$ NMR (400 MHz, DMSO-*d6*) δ 7.91 (d, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.70 (s, 1H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.13 (s, 1H), 7.11 (d, *J*

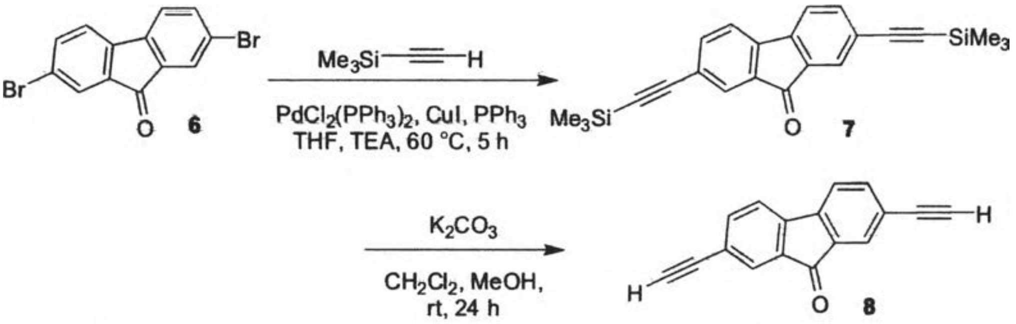
= 12.7 Hz, 1H), 2.00 (d, 2H), 1.19 - 0.86 (m, 12H), 0.74 (t, J = 6.8 Hz, 3H). $^{13}$C NMR (100 MHz, DMSO-*d6*): δ (ppm) 171.2, 160.8, 151.0, 140.78, 131.0, 130.7, 129.1, 126.1, 122.1, 120.8, 120.7, 119.3, 113.3, 93.0, 88.9, 55.1, 39.5, 31.1, 29.0, 28.41, 28.39 23.2, 22.0, 13.8. MALDI-TOF *m/z* calcd for $C_{47}H_{50}O_6$, 710.361; found, 709.670.

### 2.3.5 Preparation of 2,7-dibromo-9H-fluoren-9-one (6)



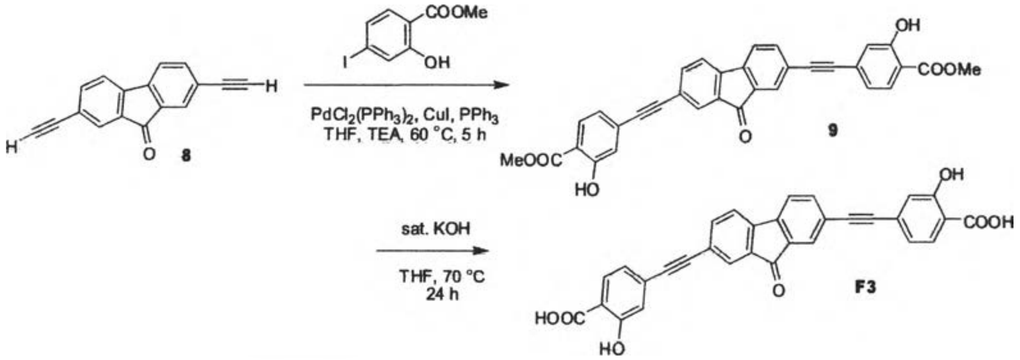
2,7-dibromofluorene (3.00 g, 9.3 mmol) and KOH (0.52 g, 9.3 mmol) was dissolved in THF (30 mL). The mixture was under oxygen atmosphere and stirred at room temperature for 5 h. The reaction was filtered to remove KOH and the filtrate was concentrated to obtain the crude product. The crude product was washed with water (3 x 20 mL) and dried to obtained yellow solid (3.3 g, 97% yield) $^1$H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, *J* = 1.3 Hz, 1H), 7.63 (d, *J* = 7.9, 1.7 Hz, 1H), 7.39 (d, *J* = 7.9 Hz, 1H). The data were agreed with those reported in literature [15].

### 2.3.6 Preparation of 2,7-diethynyl-9,9-dioctyl-9H-fluorene (8)



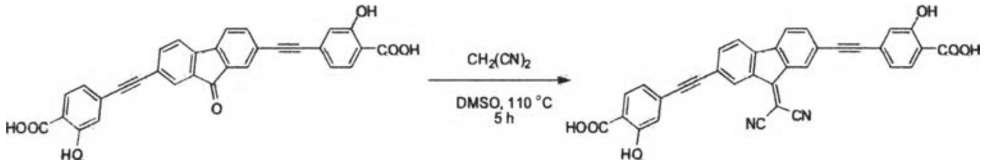
A mixture of **6** (0.50 g, 1.5 mmol), $PdCl_2(PPh_3)_2$ (0.10 g, 0.15 mmol), CuI (56 mg, 0.30 mmol), $PPh_3$ (80 mg, 0.30 mmol) and trimethylsilylacetylene (0.36 g, 3.7 mmol) in THF (15 mL) and TEA (20 mL) was added and the mixture was stirred at 60 °C for 5 h. The reaction mixture was extracted with $CH_2Cl_2$ (2 x 20 mL). The combined organic phase was washed with water (2 x 25 mL) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the crude product was purified by silica column chromatography using hexane as an eluent to afford **7** as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.45 (d, *J* = 7.7 Hz, 1H), 0.26 (s, 9H). A mixture of **7** (0.44 g, 1.2 mmol) and $K_2CO_3$ (0.68 g, 4.9 mmol) in THF (10 mL) and MeOH (25 mL) was stirred at room temperature for 24 h. The organic layer was separated and the aqueous phase was extracted with $CH_2Cl_2$ (2 x 50 mL) and was then dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the residue was eluted through a silica gel column using hexane/$CH_2Cl_2$ (4/1 v/v) as an eluent to afford **8** as a yellow solid in 68% overall yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.64 (d, $J$ = 8.0 Hz, 1H), 7.50 (d, $J$ = 7.7 Hz, 1H), 3.19 (s, 1H).

### 2.3.7 Preparation of **F3**



A mixture of **8** (0.20 g, 0.88 mmol), $PdCl_2(PPh_3)_2$ (0.12 g, 0.18 mmol), CuI (67 mg, 0.35 mmol), $PPh_3$ (92 mg, 0.35 mmol) and methyl 4-iodosalicylate (0.73 g, 2.6 mmol) in THF (15 mL) and TEA (5 mL) was added and the mixture was stirred at 60 $^{o}$C for 5 h. The reaction mixture was extracted with $CH_2Cl_2$ (2 x 20 mL). The combined organic phase was washed with water (2 x 25 mL) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the crude product was purified by silica column chromatography using hexane as an eluent to afford **9** as yellow solid. $^1$H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.61 (s, 1 H), 7.98 (d, $J$ = 8.3 Hz, 1 H), 7.88 (d, $J$ = 9.3 Hz, 1 H), 7.82 (d, $J$ = 6.2 Hz, 1 H), 7.81 (s, 1H), 7.19 (s, 1 H), 7.15 (d, $J$ = 8.4 Hz, 1 H), 3.90 (s, 3 H). To mixture of **9** (0.12 g, 0.19 mmol) in THF (10 mL) was added saturated KOH aqueous solution (0.2 mL) and the mixture was heated to 70 °C. After 24 h, the solution was evaporated and the residue was dissolved in water (20 mL). The aqueous layer was acidified by addition of 6N HCl and centrifuged. The precipitates were washed by water to neutral to afford **F3** as a brown solid in 60% overall yield. $^1$H NMR (400 MHz, DMSO-*d6*) δ 7.93 (d, $J$ = 7.8 Hz, 1H), 7.83 (d, $J$ = 7.8, 1.3 Hz, 1H), 7.75 (s, 1H), 7.70 (d, $J$ = 7.8 Hz, 1H), 6.88 (s, 1H), 6.86 (d, $J$ = 7.9 Hz, 1H). $^{13}$C NMR (100 MHz, DMSO-*d6*): δ (ppm) 171.2, 160.8, 151.0, 140.8, 131.0, 130.7, 129.1, 126.1, 122.1, 120.8, 120.7, 119.3, 113.3, 93.0, 88.9. HRMS *m/z* calcd for $C_{31}H_{16}O_7$, 500.090; found, 499.085

### 2.3.8 Preparation of F4



Compound **F3** ( 20 mg, 0.0418 mmol) and malononitrile ( 2.8 mg, 0.0418 mmol) were mixed and dissolved in DMSO. The mixture was stirred and heated up to 110 $^{o}$C for 5 h. After cooling and filtered the crude product was washed with the small amount of $CH_3CN$ to afford **F4** as a dark brown solid (14mg, 61% yield) $^1$H NMR (400 MHz, DMSO-*d6*) δ 7 8.32 (s, 1H), 8.04 (d, *J* = 7.9 Hz, 1H), 7.88 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.07 – 6.96 (m, 2H). $^{13}$C NMR (100 MHz, DMSO-*d6*): δ (ppm) 170.8, 161.8, 158.3, 141.1, 138.1, 134.2, 130.5, 128.4, 126.9, 123.3, 122.5, 121.2, 119.3, 113.2, 90.9, 89.9, 78.4. MALDI-TOF *m/z* calcd for $C_{34}H_{16}N_2O_6$, 548.101; found, 550.263.

### 2.4 Photophysical property study

The stock solutions of fluorophores at 1.0 mM were diluted to 100 µM using phosphate buffer saline (PBS, 10 mM, pH 8.0).

### 2.4.1 UV-Visible spectroscopy

The stock solutions of fluorophores were diluted to 20 µM. The UV-Visible absorption spectra of the stock solutions of fluorophores were recorded from 200 to 500 nm at ambient temperature.

### 2.4.2 Fluorescence spectroscopy

The stock solutions of fluorophores were diluted to 20 µM. The emission spectra of fluorophores were recorded from 310 to 700 nm at ambient temperature using an excitation wavelength at 300 to 330 nm, respectively.

### 2.4.3 Fluorescence quantum yields

The fluorescence quantum yield of fluorophores were performed in phosphate buffer saline (PBS, 10 mM) pH 8.0 using quinine sulphate ($\Phi_F$ = 0.54) in 0.1 M $H_2SO_4$ as a reference [16] The maximum absorbance of all samples should not exceed 0.1. The fluorescence emission spectra of the same solutions using appropriate excitation wavelengths selected were recorded based on the absorption maximum wavelength ($\lambda_{max}$) of each compound. Graphs of integrated fluorescence intensities were plotted against the absorbance at the respective excitation wavelengths. Each plot should be linear with a y-interception at 1 and gradient m [17]

In addition, the fluorescence quantum yield ($\Phi_F$) was obtained from a plot between integrated fluorescence intensity and absorbance as represented in the following equation:

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

The subscripts $\Phi_{ST}$ denotes the fluorescence quantum yield of a standard reference which used quinine sulphate in 0.1 M $H_2SO_4$ ($\Phi_F = 0.54$) and $\Phi_X$ is the fluorescence quantum yield of sample and $\eta$ is the refractive index of the solvent.

### 2.5 Fluorescent sensor study

#### 2.5.1 Metal ion sensor

The stock solutions of fluorphores with a concentration of 1 mM in phosphate buffer saline pH 8.0 were prepared. The emission spectrum of compound was recorded from 310 to 700 nm at ambient temperature using an excitation wavelength in the range of 300-340 nm. The fluorescent responses were monitored in the presence of 17 metal ions, with and without surfactants. Metal acetate, sulphate, nitrate and chloride solutions were prepared in Milli-Q water. Concentrations of all stock metal ion solutions were adjusted to 1 mM and were added with the desired volumes (0-500 µL) to the fluorophore solutions. The final volumes of the samples were adjusted to 1.0 mL.

#### 2.5.2 Surfactant enhancement

The stock solution of compound **F2** with a concentration of 10 µM in phosphate buffer saline pH 8.0 was prepared. The emission spectrum of **F2** was recorded from 330 to 600 nm at ambient temperature using an excitation wavelength at 320 nm. The photophysical properties were studied in the presence of three types of surfactants (anionic, cationic and non-ionic surfactants). The stock surfactants were prepared in Milli-Q water. Concentrations of all stock surfactants were adjusted to 1 mM and were added with the desired volumes (0-100 µL) to the fluorophore solutions. The final volumes of the samples were adjusted to 1.0 mL.