CHAPTER II

Experiment

2.1 Chemical and Materials

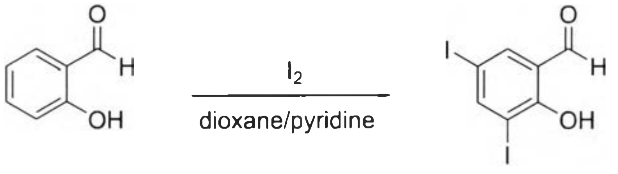
Bis(triphenylphosphine)palladium(II)dichloride ($PdCl_2(PPh_3)_2$), sodium thiosulfate trimethylsilylacetylene, triphenylphosphine, 2,4-hydroxybenzaldehyde and potassium carbonate were purchased from Fluka. Copper (I) iodide, 1, 8-diazabicyclo [5.4.0] undec-7-ene (DBU), hydroquinone, butylbromide, octylbromide and 2-hydroxybenzaldehyde were purchased from Aldrich. All other reagents were non-selectively purchased from Sigma-Aldrich, Fluka or Merck and used without further purification. For most reactions, solvents such as methylene chloride and acetonitrile were reagent grade stored over molecular sieves. In anhydrous reactions, solvents such as THF and toluene were dried and distilled before use according to the standard procedures. All 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). Solvents used for extraction and chromatography such as dichloromethane, hexane, ethyl acetate and methanol were commercial grade and distilled before use while diethyl ether and chloroform were reagent grade. Milli-Q water was used in all experiments unless specified otherwise. The most reactions were carried out under positive pressure of N_2 filled in rubber balloons.

2.2 Analytical Instruments

The ^{1}H-NMR and ^{13}C-NMR spectra were acquired from sample solution in $CDCl_3$, D_2O and DMSO-d_6 on a Varian Mercury NMR spectrometer, which operate at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR. Mass spectra were obtained by electrospray ionization mass spectrometry (ESI). Absorption spectra were measured by a Varian Cary 50 UV-Vis spectrophotometer. Emission spectra were obtained from a Varian Cary Eclipse spectrofluorometer. Fourier transform infrared spectra were acquired on Nicolet 6700 FT-IR spectrometer equipped with a mercury-cadmium telluride (MCT) detector (Nicolet, USA). All polymer solutions were filtered through 0.45 μm syringe filters prior to use. Polymer molecular weights were determined at 25 °C on a HP series 1100 GPC system in THF at 1.0 mL/min (3mg/mL sample concentrations) equipped with a diode array detector (254 nm and 450 nm). Polymer molecular weights are reported relative to polystyrene standards.

2.3 Synthesis of monomer

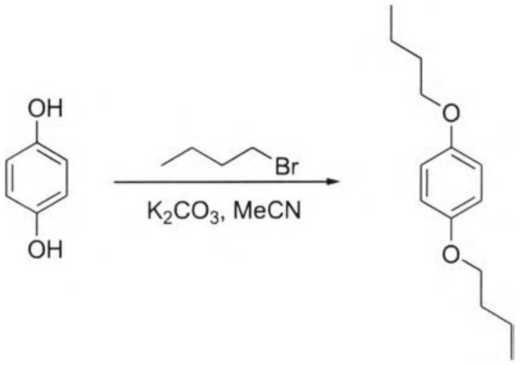
2.3.1 Synthesis of diiodosalicylaldehyde (2a)



Scheme 2.1 Synthesis of **2a**

Iodine (38.1 g, 0.15 mol) was dissolved by the mixture solution of pyridine and dioxane (1:1) in the round bottom flask. Then, the reaction mixture was stirred by using magnetic on ice bath for 15 minutes. Salicylaldehyde (5.24 mL, 0.5 mol) was added into the reaction flask. This mixture was left to react overnight. The reaction mixture was extracted with CH_2Cl_2 mixed with water. The organic layer was washed with $Na_2S_2O_3$ and HCl, respectively, followed by evaporation of the solvent. Finally, the reaction was precipitated with CH_2Cl_2 to provide product to afford 5.18 g (13.85 mmol) of 2a as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 11.75 (s, 1H), 9.69 (s, 1H), 8.31 (s, 1H), 7.84(s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 194.77, 160.04, 152.82, 142.16, 121.84, 87.37, 81.39.

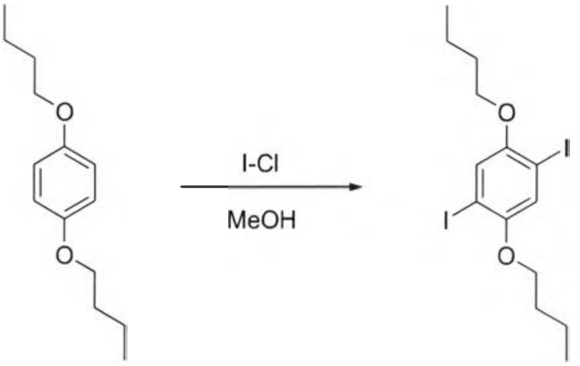
2.3.2 Synthesis of dibutoxybenzene (2c)



Scheme 2.2 Synthesis of **2c**

The suspension of KOH (4.58 g, 81.72 mmol) and hydroquinone (2.00 g, 18.16 mmol) in DMF (15 mL) was stirred, then n-butyl bromide (9.95 g, 72.64 mmol) was gradually added. The mixture was stirred at room temperature overnight. The mixture was poured into 300 mL of cool water and the organic layer was filtered, washed with cool water many times, and dried over a steam bath to afford 8.82 g (18.16 mmol, 76%) of 1a as a light brown solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.82 (s, 4H), 3.92-3.89 (t, 4H), 1.77-1.70 (m, 4H), 1.52-1.43 (m, 4H), 0.98-0.94 (t, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 153.7, 115.9, 68.9, 32.0, 19.7, 14.3.

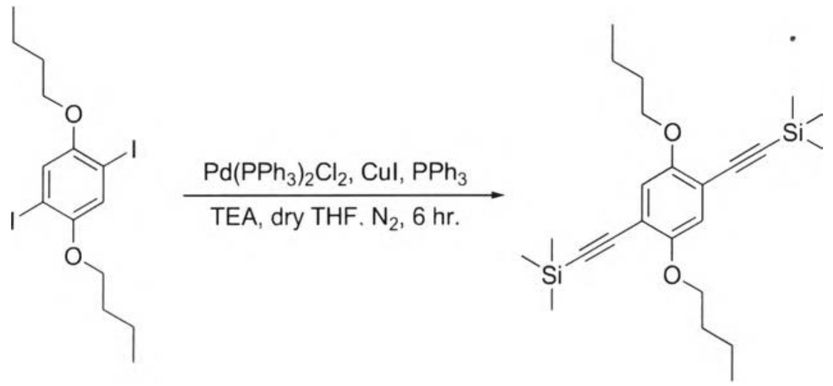
2.3.3 Synthesis of 1,4-dibutoxy-2,5-diiodobenzene (3c)



Scheme 2.3 Synthesis of **3c**

The 1,4-dibutylbenzene (**2c**) (4.00 g, 17.99 mmol) was stirred in MeOH (30 mL) at temperature below 15 $^{\circ}$C. Then I-Cl (12.76 g, 78.61 mmol) was gradually added. The mixture was stirred reflux for 4 hr. Methylenechloride was used to extract the mixture for 3 times. The extract part was washed with aqueous Na2S2O3, water, and brine. After that the substance was dried over anhydrous anhydrous Na2SO4, concentrated in vacuo and purified by column chromatography to provide product **3c** to afford 6.25 g (17.99 mmol, 74%) of 2a as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.17 (s, 2H), 3.95-3.92 (t, 4H), 1.82-1.75 (m, 4H), 1.58-1.49 (m, 4H), 1.00-0.96 (t, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 153.2, 123.2, 86.6, 70.4, 31.6, 19.6, 14.1

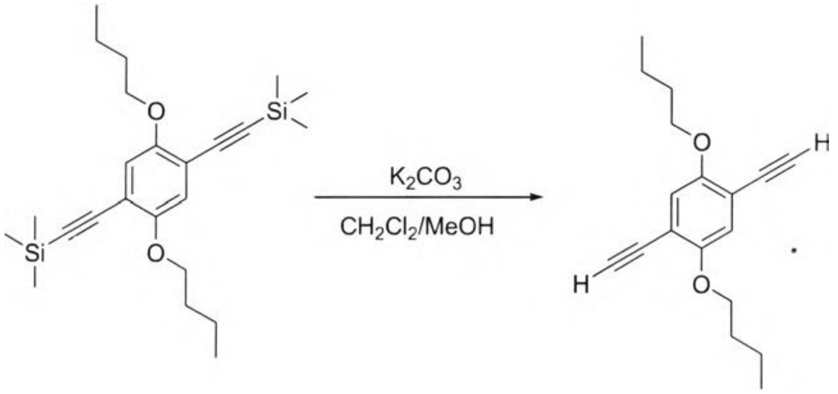
2.3.4 Synthesis of 1,4-dibutoxy-2,5-bis(2-(trimethylsilyl)ethynyl)benzene (4c)



Scheme 2.4 Synthesis of **4c**

1,4-dibutoxy-2,5-diiodobenzene (**3c**) (0.7g, 2.82×10^{-3} mol) was mixed with $Pd(Ph_3)_2Cl_2$ (0.040 g, 5.64×10^{-5} mol), CuI (0.021, 1.128×10^{-4} mol) and PPh_3 (0.03 g, 1.128×10^{-4} mol) in the bottom flask under N_2 atmosphere. Then, THF and TEA were added to the flask and stirred at room temperature for 15 min. After that, ethynyltrimethylsilane (0.83 mL, 5.64×10^{-3} mol) was dropped and the solution was left for 6 hr. Next, the solvent was evaporated. The residue was purified by column chromatography on silica gel (100% hexane). Finally, the evaporation of solvent was done to receive a white solid **4c** in 68% (0.79 g, 19 mmol). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 6.89 (s, 2H), 3.95 (t, J = 6.3 Hz, 4H), 1.80 – 1.73 (m, 4H), 1.56 – 1.49 (m, 4H), 0.97 (t, J = 7.4 Hz, 6H), 0.25 (s, 18H).

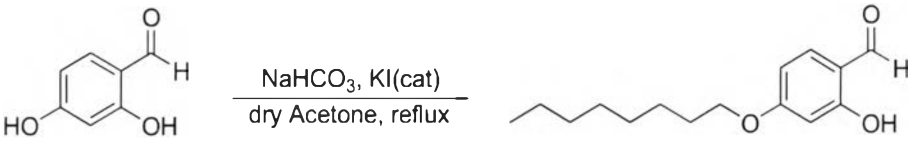
2.3.5 Synthesis of 1,4-dibutoxy-2,5-diethynylbenzene (5c)



Scheme 2.5 Synthesis of **5C**

1,4-dibutoxy-2,5-bis(2-(trimethysilyl)ethynyl)benzene (**4c**) was added into a 100 mL round bottom flask containing a magnetic bar. The 20% K_2CO_3 (35 mg, 0.25 mmol), CH2Cl2 (5 mL) and MeOH (5 mL) were added into the flask. The reaction mixture was stirred under N_2 at rt for 4 h. The mixture was extracted three times with CH_2Cl_2. The combined organic phase was washed three times by NH_4Cl and one time by water, respectively. Then, the organic solution was dried over anhydrous Na2SO4, concentrated in vacuo and purified by column chromatography to provide 1,4-dibutoxy-2,5-diethynylbenzene (**5c**) 0.28 g (1.05 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.95 (s, 2H), 3.98 (t, J = 6.5 Hz, 4H), 3.33 (s, 2H), 1.82 – 1.75 (m, 4H), 1.56 – 1.47 (m, 4H), 0.97 (t, J = 7.4 Hz, 6H).

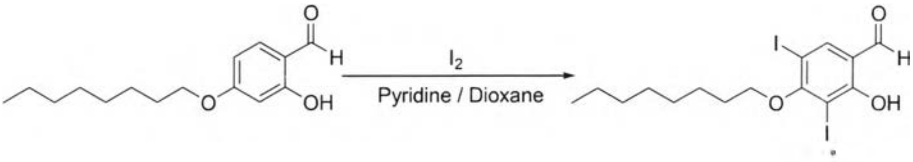
2.3.6 Synthesis of 8-octylsalicylaldehyde



Scheme 2.6 Synthesis of **2b**

The mixture of KOH (4.58 g, 81.72 mmol) and 4-hydroxy salicylaldehyde (**1b**) (2.00 g, 18.16 mmol) in acetone (15 mL). Then n-octylbromide (9.95 g, 72.64 mmol) was gradually added stirred at 60°C overnight. The mixture was poured into 300 mL of cool water and the organic layer was filtered, washed with cool water many times, and dried over a steam bath to afford 8.82 g (18.16 mmol, 76%) of **2b** as a light brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.47 (s, 1H), 9.68 (s, 1H), 7.39 (d, J = 8.6 Hz, 1H), 6.56 – 6.32 (m, 2H), 3.98 (t, J = 6.6 Hz, 2H), 1.86 – 1.69 (m, 2H), 1.43 (s, 2H), 1.29 (d, J = 11.2 Hz, 8H), 0.88 (t, J = 6.7 Hz, 3H).

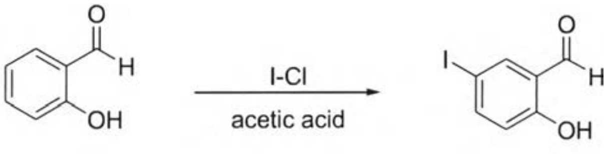
2.3.7 Synthesis of 3,5-diiodo-4-octylsalicylaldehyde



Scheme 2.7 Synthesis of **3b**

Iodine (3.81 g, 0.015 mol) was dissolved by the mixture solution of pyridine and dioxane (1:1) in the round bottom flask. Then, the reaction mixture was stirred by using magnetic on ice bath for 15 minutes. 4-octyl salicylaldehyde (**2b**) (5.24 mL, 0.5 mol) was added into the reaction flask. This mixture was left to react overnight. The reaction mixture was extracted with CH_2Cl_2 mixed with water. The organic layer was washed with $Na_2S_2O_3$ and HCl, respectively, followed by evaporation of the solvent. Finally, the reaction was precipitated with CH_2Cl_2 to provide product to afford of 3,5-diiodo-4-octylsalicylaldehyde (**3b**) as a yellow solid 5.18 g (13.85 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.02 (s, 1H), 9.67 (s, 1H), 7.96 (s, 1H), 4.04 (t, J = 6.5 Hz, 2H), 2.01 – 1.90 (m, 2H), 1.55 (d, J = 8.2 Hz, 3H), 1.46 – 1.22 (m, 9H), 0.88 (d, J = 7.0 Hz, 3H).

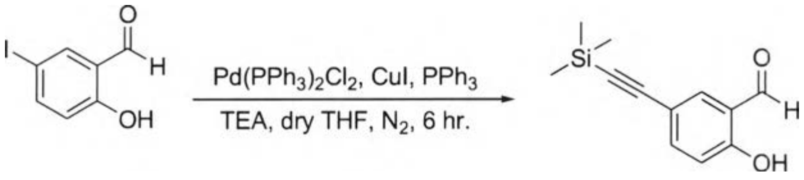
2.3.8 Synthesis of 5-iodosalicylaldehyde (1d)



Scheme 2.8 Synthesis of **1d**

Salicylaldehyde (**1a**) (0.087mL, 8.19×10^{-4} mol) was dissolved in acetic acid, and followed by addition of iodine monochloride (0.046 ml , x mol) at room temperature. The reaction mixture was left overnight. After the reaction was completed, the reaction mixture was extracted with CH_2Cl_2 and $NaHCO_3$, respectively. And, the solvent was removed by evaporator. The residue was purified by column chromatography on silica gel (5% EtOAc) in hexane). Last, the solvent evaporation was done to get a mild yellow-colored powder (**1d**) 0.126 g (5 mmol, 62%). ^1H NMR (400 MHz, $CDCl_3$) δ 10.94 (s, 1H), 9.82 (s, 1H), 7.84 (s, 1H), 7.76 (d, J = 8.8 Hz, 1H), 6.79 (d, J = 8.8 Hz, 1H). ^{13}C NMR (101 MHz, CDCl3) δ ppm 195.27, 161.27, 145.47, 141.98, 122.69, 120. 09, 80.38.

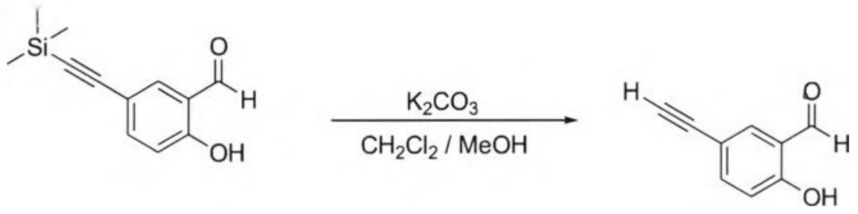
2.3.7 Synthesis of 2-hydroxy-5-(triethynylsilyl)benzaldehyde (2d)



Scheme 2.9 Synthesis of **2d**

5-diiodosalicylaldehyde (**1d**) (0.7g, 2.82×10^{-3} mol) was mixed with $Pd(Ph_3)_2Cl_2$ (0.040 g, 5.64×10^{-5} mol), CuI (0.021, 1.128×10^{-4} mol) and PPh_3 (0.03 g,1.128×10^{-4} mol) in the bottom flask under N_2 atmosphere. Then, THF and TEA were added to the flask and stirred at room temperature for 15 min. After that, ethynyltrimethylsilane (0.83 mL, 5.64×10^{-3} mol) was dropped and the solution was left for 6 hr. Next, the solvent was evaporated. The residue was purified by column chromatography on silica gel (10% EtOAc) in hexane). Finally, the evaporation of solvent was done to receive a yellow-colored powder **2d** 0.23 g (10 mmol, 37%). ^1H NMR (400 MHz, CDCl3) δ 11.11 (s, 1H), 9.85 (s, 1H), 7.71 (s, 1H), 7.60 (d, J = 8.4 Hz, 1H), 6.93 (d, J = 8.6 Hz, 1H), 0.25 (s, 9H).

2.3.8 Synthesis of 2-hydroxy-5-ethynylbenzaldehyde (3d)

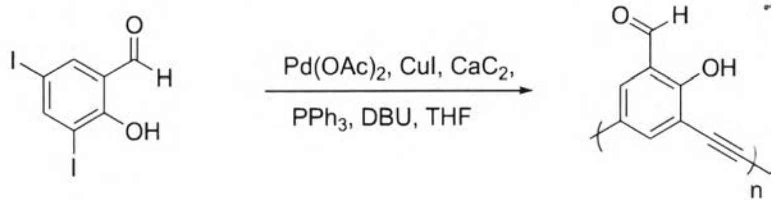


Scheme 2.10 Synthesis of **3d**

A 100 mL round bottom flask with a magnetic stir bar was charged with 2-hydroxy-5-(triethynylsilyl)benzaldehyde (**2d**) (0.52 g, 1.26 mmol). The mixture was added with 20% K_2CO_3 (35 mg, 0.25 mmol), methylene chloride (5 mL) and MeOH (5 mL). The reaction mixture was stirred under N_2 at room temperature for 4 h. The mixture was extracted three times with methylene chloride. The combined organic phase was washed three times with NH4Cl and washed again with H_2O and then dried over anhydrous Na_2SO_4, concentrated in vacuo and purified by column chromatography to provide 0.28 g (1.05 mmol, 83%) of 2-hydroxy-5-ethynylbenzaldehyde (**3d**) as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ 11.13 (s, 1H), 9.87 (s, 1H), 7.72 (s, 1H), 7.62 (d, J = 8.7 Hz, 1H), 6.96 (d, J = 8.6 Hz, 1H), 3.04 (s, 1H).

2.4 Synthesis of Poly(phenylene ethynylene)

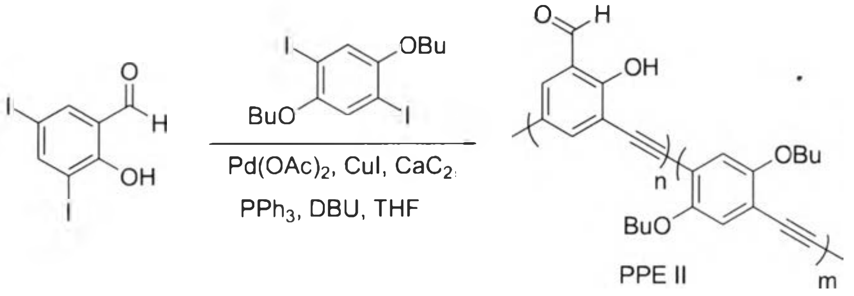
2.4.1 Synthesis of PPE I



Scheme 2. 11 Synthesis of **PPE I**

A 100 mL round bottom flask with a magnetic stirrer bar was charged with 3,5-diiodosalicylaldehyde (116 mg, 0.31 mmol), palladium(II)acetate (3.37 mg, 0.015 mmol), copper iodide (5.90 mg, 0.031 mmol), triphenylphosphine (8.13 mg, 0.031 mmol), calcium carbide (119.22 mg, 1.86 mmol) and DBU (2 mL) in THF (4 mL). The reactions were carried out under pressure of N_2 filled in rubber balloons. The mixture was stirred at room temperature for 20 hours. The solution was then filtrated with cotton wool by methylene chloride as eluent, concentrated to a small volume and precipitated by dropping the solution into 150 mL of methanol. The precipitate that formed was collected by centrifuge, washed repeatedly with methanol and evaporated under vacuum to afford 44.64 mg (quantitative yield) of **PEE I** as a dark solid.

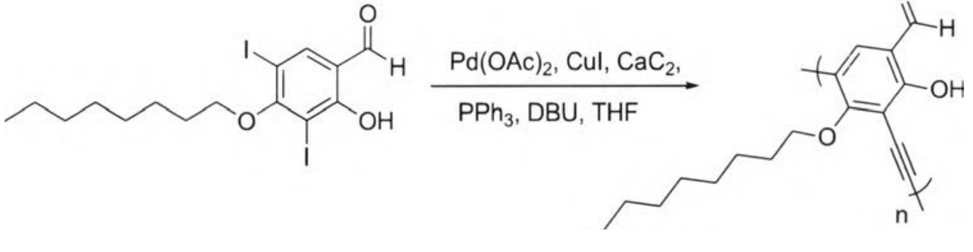
2.4.2 Synthesis of PPE III



Scheme 2.12 Synthesis of **PPE III**

A 100 mL round bottom flask with a magnetic stirrer bar was charged with 3,5-diiodosalicylaldehyde (58 mg, 0.16 mmol) and 1,4-dibutoxy-2,5-diiodobenzene (73 mg, 0.16 mmol), palladium(II)acetate (3.37 mg, 0.015 mmol), copper iodide (5.90 mg, 0.031 ımol), triphenylphosphine (8.13 mg, 0.031 mmol), calcium carbide (119.22 mg, 1.86 mmol) and DBU (2 mL) in THF (4 mL). The reactions were carried out under positive pressure of N_2 filled in rubber balloons. The mixture was stirred at room temperature for 20 hours. The solution was then filtrated with cotton wool by methylene chloride as eluent, concentrated to a small volume and precipitated by dropping the solution into 150 mL of methanol. The precipitate that formed was collected by centrifuge, washed repeatedly with methanol and evaporated under vacuum to afford 121 mg (quantitative yield) of **PEE II** as a dark solid. $M_w = 9.5 \times 10^3 - 1.86 \times 10^4$..

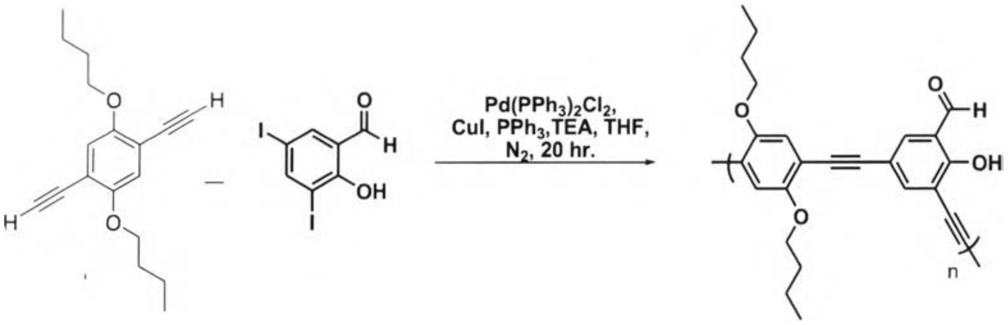
2.4.3 Synthesis of PPE II



Scheme 2.13 Synthesis of **PPE II**

A 100 mL round bottom flask with a magnetic stirrer bar was charged with 3,5-diiodo-4-octylsalicylaldehyde (116 mg, 0.31 mmol), palladium(II)acetate (3.37 mg, 0.015 mmol), copper iodide (5.90 mg, 0.031 mmol), triphenylphosphine (8.13 mg, 0.031 mmol), calcium carbide (119.22 mg, 1.86 mmol) and DBU (2 mL) in THF (4 mL). The reactions were carried out under pressure of N_2 filled in rubber balloons. The mixture was stirred at room temperature for 20 hours. The solution was then filtrated with cotton wool by methylene chloride as eluent, concentrated to a small volume and precipitated by dropping the solution into 150 mL of methanol. The precipitate that formed was collected by centrifuge, washed repeatedly with methanol and evaporated under vacuum to afford 44.64 mg (quantitative yield) of **PEE III** as a dark solid. $M_w = 9.5x10^3$.

2.4.4 Synthesis of PPE IV

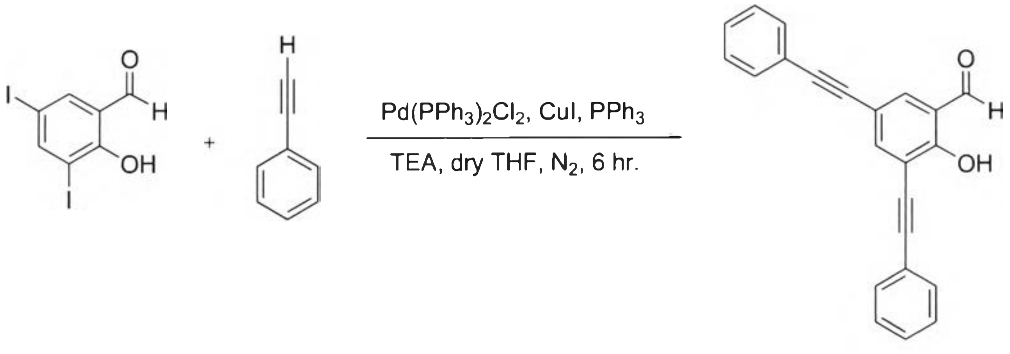


Scheme 2.14 Synthesis of **PPE IV**

3,5 diiodosalicylaldehyde (**2a**) (0.1g, $2,64\times10^{-4}$ mol) was mixed with $Pd(Pph_3)_2Cl_2$ (0.0033 g, 1.34×10^{-5} mol), CuI (0.005 g, 2.6×10^{-5} mol) and PPh_3 (0.0069 g, 2.6×10^{-5} mol) in round bottom flask under N_2 atmosphere. After that, THF and TEA were added to the flask and stirred for 15 min. Then, 1,4-dibutoxy-2,5-diethynylbenzene (**5c**) (0.086 mL, 7.8×10^{-4} mol) was added. The mixture was stirred at room temperature for 20 hours. The solution was then filtrated with cotton wool by methylene chloride as eluent, concentrated to a small volume and precipitated by dropping the solution into 150 mL of methanol. The precipitate that formed was collected by centrifuge, washed repeatedly with methanol and evaporated under vacuum to afford 44.64 mg (87%) of **PEE IV** as a dark solid. $M_w = 6.5x10^3$.

2.5 Synthesis of phenylene ethynylene

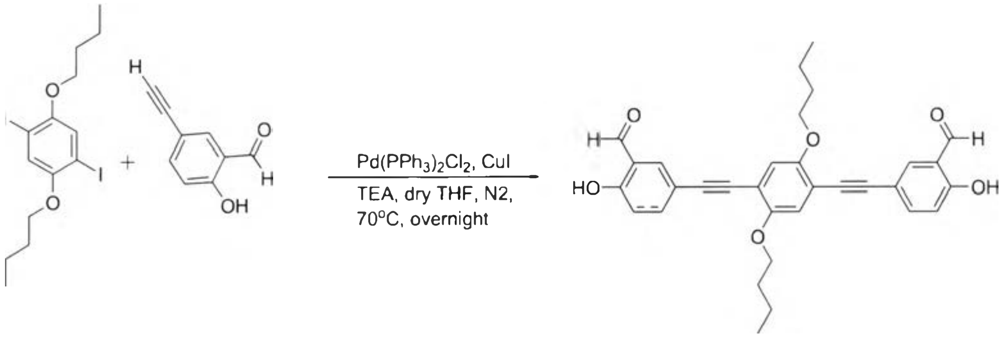
2.5.1 Synthesis of PE I



Scheme 2. 15 Synthesis of PE I

3,5 diiodosalicylaldehyde (0.1g, 2,64x10^{-4} mol) was mixed with Pd(Pph$_3$)$_2$Cl$_2$ (0.0033 g, 1.34x10^{-5} mol), CuI (0.005 g, 2.6x10^{-5} mol) and PPh$_3$ (0.0069 g, 2.6x10^{-5} mol) in round bottom flask under N2 atmosphere. After that, THF and TEA were added to the flask and stirred for 15 min. Then, phenylacetylene (0.086 mL, 7.8x10^{-4} mol) was gradually dropped. The mixture was left overnight. The rotary evaporator was used to evaporate solvent from the mixture. The residue was purified by column chromatography on silica gel (10% EtOAc in hexane). Finally, the mixture was precipitated with EtOAc. ^{1}H NMR (400 MHz, CDCl3) δ 10.56 (s, 1H), 7.98 (d, J = 7.4 Hz, 2H), 7.93 (d, J = 7.2 Hz, 2H), 7.56 (d, J = 3.3 Hz, 2H), 7.50 (t, J = 7.4 Hz, 2H), 7.43 (d, J = [illegible].3 Hz, 1H), 7.41 – 7.34 (m, 3H), 7.09 (s, 1H). 13C NMR (400 MHz, CDCl$_3$) δ ppm 187.45, 158.33, 153.57, 131.66, 131.18, 129.50, 128.94, 128.45, 125.24, 123.02, 120.88, 118.73, 100.48, 89.26, 88.40.

2.5.2 Synthesis of PE II



Scheme 2.16 Synthesis of **PE II**

5-ethylnylsalicylaldehyde (100 mg, 0.68 mmol) was mixed with 1,4-bis-butyl-2,5-diiodobenzene (159 mg, 0.228 mmol), $Pd(Pph_3)_2Cl_2$ (24 mg, 0.034 mmol) and CuI (6 mg, 0.032 mmol). This reaction was dissolved in THF (5 mL). After that, TEA (20 mL) was added and the mixture was heated to 70 °C overnight. Subsequent to cooling down to room temperature, a rotary evaporator was used to remove the solvent of the reaction. Flash chromatography was used to purify the residue on silica gel (EtOAc: Hexane, 1:3). Then EtOAc was used as eluent on short plug of silica gel. Removing and washing of the solution was done by using methanol to get **PE II** as a yellow powder 145 mg (0.286 mmol, 42%). ^1H NMR (400 MHz, $CDCl_3$) δ 11.13 (s, 1H), 9.89 (s, 1H), 7.75 (s, 1H), 7.67 (d, J = 10.1 Hz, 1H), 7.00 (d, J = 5.6 Hz, 2H), 4.04 (t, J = 6.4 Hz, 2H), 1.92 – 1.77 (m, 2H), 1.66 – 1.48 (m, 2H), 1.01 (t, J = 7.3 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 187.45, 158.33, 153.57, 131.66, 131.18, 129.50, 129.20, 128.94, 128.48, 128.24, 123.02, 120.88, 118.73, 100.48, 89.26, 88.40 [41].

2.6 Photophysical properties

The stock solutions of 0.5 mM fluorophore in DMSO was prepared.

2.6.1 UV-Visible spectroscopy

The UV-Visible absorption spectra of the stock solution of fluorophore were recorded from 250 nm to 600 nm at ambient temperature

2.6.2 Fluorescence spectroscopy

The stock solution of 0.5 mM fluorophore were diluted to 5 μM in 90% DMSO in 10 μM HEPES buffer pH 7.4. The emission spectra of fluorophore were recoreded from 350 to 650 nm at ambient temperature using an excitation wavelength at 340 to 460 nm, respectively.

2.6.3 Fluorescence quantum yield

The fluorescence quantum yield of fluorophores were performed in DMSO and HEPES buffer (10 mM) pH 7.4 by using quinine sulphate (ΦF = 0.54) in 0.1 H_2SO_4 as a reference. The UV-Visible absorption spectra of five analytical samples and five reference samples at varied concentrations were recorded. The maximum absorbance of all samples should never exceed 0.1. The fluorescence emission spectra of the same solutions using appropriate excitation wavelengths selected were recorded based on the absorption maximum wavelength (λmax) of each compound. Graphs of integrated fluorescence intensities were plotted against the absorbance at the respective excitation wavelengths. Each plot should be a straight line with 1 interception and gradient m [42]. In addition, the fluorescence quantum yield (ΦF) was obtained from plotting of integrated fluorescence intensity vs absorbance represented into 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 Φ ST denote the fluorescence quantum yield of a standard reference which used quinine sulfate in 0.1 M H2SO4 (Φ = 0.54) and Φ X is the fluorescence quantum yield of sample and η is the refractive index of the solvent

2.7 Fluorescence sensor study

2.7.1 Anion sensor

The excitation wavelength were 341 nm, 365 nm, 457 nm, 417 nm, 315 nm, 369 nm, for PPE I, PPE II, PPE III(c), PPE IV, PE I, and PE II and the emission were record from 360-700 nm. The anions solution were prepared in Milli-Q water. Concentration of all anion solutions were adjust to 10 mM and were added with the desired volumes (15 μL) to the fluorophore solutions. The final volumes of the mixture were adjusted to 1000 μL to afford the final concentration of 5 μM for the fluorophoe and 150 μM for anions.

2.7.2 Surfactant study

The excitation wavelength was 315 nm and the emission was recorded from 325-600 nm. Surfactant solutions were prepeared in Milli-Q water. Concentration of all stock anion solutions were adjusted to 10 mM and were added with the desired volume (10 mL) to fluorophore solutions. The final volumes of the mixture were adjusted to 1000 μL to afford the final comcentration of 5 μM for the fluorophore and 10 μM for surfactant.