CHAPTER II

EXPERIMENTS

2.1 Chemicals

Thin layer Chromatography (TLC) was performed on aluminum sheets precoated with silica gel (Merck Kieselgel 60 F_{254}, Merck KGaA, Darmstadt, Germany). Column chromatography was performed using 0.063-0.200 mm or 70-230 mesh ASTM silica gel 60 (Merck Kieselgel 60 G, Merck KGaA, Darmstadt, Germany). Solvents used in synthesis were reagent or analytical grades. Solvents used in column chromatography were distilled from commercial grade prior to use. Other reagents were purchased from the following venders:

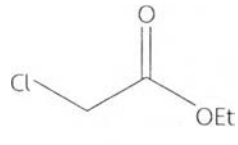
- RCI Labscan (Bangkok, Thailand): acetone, acetonitrile, dichloromethane, nitric acid (HNO_3), chloroform, dimethylsulfoxide (DMSO), dimethylformamide (DMF), sodium hydrogen carbonate ($NaHCO_3$)
- Acrös Organics (New Jersey, USA): dimethyl sulfate ($(CH_3)_2SO_4$), quinoline, 1,2-dibromoethane, *N*-bromosuccinimide (NBS), *p*-toluenesulfonic acid (PTSA)
- Carlo Erba (Milan, Italy): triethylamine (TEA), fuming nitric acid
- Fluka Chemical (Buchs, Switzerland): cuprous oxide (Cu_2O), sodium metal, potassium carbonate (K_2CO_3)
- Merck Co. (Darmstadt, Germany): chloroacetyl chloride, sodium hydroxide (NaOH), ethanol absolute (EtOH), diethyl ether (Et_2O), concentrated hydrochloric acid, concentrated sulfuric acid, acetic acid (AcOH), glycerol, epichlorohydrin, *p*-nitrophenol
- Ajax Finechem Pty (Auckland, New Zealand): calcium chloride
- Cambridge Isotope Laboratories (USA): deuterated chloroform ($CDCl_3$), deuterated dimethylsulfoxide (DMSO-d_6)
- Aldrich (USA): ethyl chloroacetate, diethyl oxalate $(CO_2Et)_2$, 3,4-ethylene-dioxythiophene (EDOT), 3,4-dimethoxythiophene (DMT), deuterium oxide (D_2O), anhydrous magnesium sulfate ($MgSO_4$)

2.2 Instruments and equipment

Melting points were determined with a Stuart Scientific Melting Point SMP10 (Bibby Sterlin Ltd., Staffordshire, UK). The FT-IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer. The ^{1}H and ^{13}C NMR spectra were obtained in deuterated chloroform ($CDCl_3$), deuterated dimethylsulfoxide (DMSO-d_6) or deuterium oxide (D_2O) using Varian Mercury NMR spectrometer operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C nuclei (Varian Company, USA). The mass spectra were recorded on Waters Micromass Quatto micro API ESCi (Waters, USA). Samples were dissolved in EtOAc, MeOH, acetone or water. The UV-Vis absorption spectra were recorded on an Agilent 8453E UV-Visible spectroscopy. Gas chromatography was recorded on Varian CP-3800 GC.

2.3 Monomer synthesis

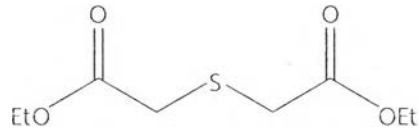
2.3.1 Ethyl chloroacetate 1



1

50 mL (56.510 g, 0.50 mol) of chloroacetyl chloride was slowly dropped into 32 mL (25.4 g, 0.55 mol) of EtOH over period of 30 min. The reaction mixture was stirred at 0 °C and then warmed to room temperature and stirred for another 3 h. The mixture was quenched by adding 120 mL of 2 M NaOH. The organic layer was separated and the aqueous layer was extracted with diethyl ether three times. The combined organic layers were dried over anhydrous $MgSO_4$. The solvent was removed using rotary evaporator to give an almost quantitative yield of **1** as colorless liquid (55 mL, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.24 (q, J = 7.2 Hz, 2H), 4.04 (s, 2H), 1.29 (t, J = 7.2 Hz, 3H) **(Figure A.1, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 167.3, 62.2, 40.9, 14.0 **(Figure A.2, Appendix A)**.

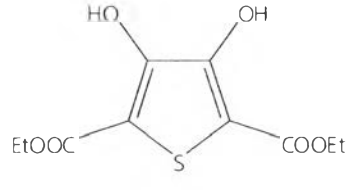
2.3.2 Diethyl thiodiglycolate 2



2

Solution of sodium sulfide nonahydrate ($Na_2S.9H_2O$, 12.0 g, 50 mmol) in water (30 mL) was added dropwise to the solution of compound **1** (13.240 g, 55 mmol) in acetone (50 mL). The reaction was stirred and refluxed for 3 h under nitrogen atmosphere. After cooling back to room temperature, the organic layer was separated and the aqueous layer was extracted with diethyl ether three times. The combined organic layers were dried over anhydrous $MgSO_4$ and then evaporated by a rotary evaporator to give compound **2** as a yellow liquid (5.030 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.19 (q, *J* = 7.1 Hz, 4H), 3.37 (s, 4H), 1.28 (t, *J* = 7.2 Hz, 6H) **(Figure A.3, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 169.5, 61.1, 33.3, 13.9 **(Figure A.4, Appendix A)**. MS: $[M+Na]^+$ m/z = 229.05 **(Figure A.5, Appendix A)** [34].

2.3.3 Diethyl 3,4-dihydroxythiophene-2,5-dicarboxylate (DDTD)

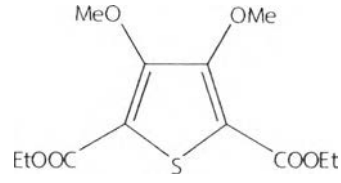


DDTD

Sodium metal (4.0 g, 0.35 mol) was dissolved in EtOH (75 mL) and then added dropwise to a mixture of compound **2** (4.217 g, 0.021 mol) and diethyl oxalate (7.2 g, 0.05 mol) over 30 min in ice bath. The reaction mixture was refluxed for additional 3 h under nitrogen, cooled to room temperature, poured into water (400 mL) and acidified by concentrated hydrochloric acid (15 mL) to afford a yellow precipitate. The filtered solid was recrystallized from methanol to give DDTD as white needle crystals (3.502 g, 68%). mp. 134-135 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.36 (s, 2H), 4.39 (q, *J* = 7.1 Hz, 4H), 1.38 (t, *J* = 7.1 Hz, 6H) **(Figure A.6, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 165.5, 151.6, 107.1, 61.7, 14.0 **(Figure A.7,**

Appendix A). IR (ATR, cm^{-1}): 3305 (-OH st), 2981 (-CH st), 1690 (C=O st), 1663 (C=C st) **(Figure A.8, Appendix A)**. MS: $[M+H]^+$ m/z = 250.20 **(Figure A.9, Appendix A)** [35].

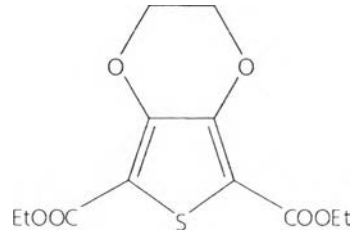
2.3.4 Diethyl 3,4-dimethoxythiophene-2,5-dicarboxylate 3a



3a

Compound DDTD (0.262 g, 1.0 mmol), dimethyl sulfate (0.28 mL, 3 mmol) and K_2CO_3 (1.38 g, 10 mmol) were mixed in acetonitrile (30 mL). The reaction was brought to reflux for 3 h under nitrogen atmosphere. After completion, the reaction mixture was added water until all precipitate completely dissolved, and then acidified by concentrated hydrochloric acid (10 mL). The mixture was extracted with ethyl acetate and the organic layer was separated. The aqueous layer was extracted with ethyl acetate three times. The collected organic layer was dried over anhydrous $MgSO_4$ and evaporated using rotary evaporator. The obtained crude was then purified by column chromatography using hexane:ethyl acetate (2:1) as eluent to yield the product as a light yellow solid (0.210 g, 73%). mp. 55-57 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.35 (q, J =7.1 Hz, 2H), 4.01 (s, 3H), 1.38 (t, J = 7.1 Hz, 3H) **(Figure A.10, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 160.5, 154.0, 119.4, 61.9, 61.4, 14.2 **(Figure A.11, Appendix A)**. IR (ATR, cm^{-1}): 2980, 2943 (-CH st), 1703 (C=O st), 1027 (-C-O st) **(Figure A.12, Appendix A)** [36].

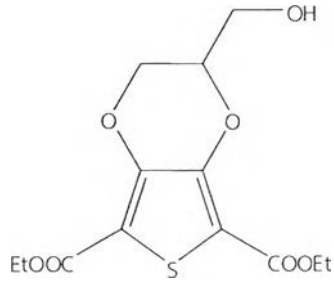
2.3.5 Diethyl 2,3-dihydrothieno[3,4-*b*]-1,4-dioxine-5,7dicarboxylate 3b



3b

Compound DDTD (0.260 g, 1.0 mmol), 1,2-dibromoethane (0.48 mL, 5.5 mmol) and triethylamine (0.77 mL, 5.5 mmol) were dissolved in acetonitrile (10 mL). The mixture was allowed to stir for 24 h at reflux temperature under nitrogen atmosphere. The reaction mixture was quenched by adding 10% hydrochloric acid solution. Then the organic layer was separated and the aqueous layer was extracted with ethyl acetate three times. The collected organic extract was dried over anhydrous $MgSO_4$ and evaporated using rotary evaporator. The crude mixture was purified by column chromatography eluted with ethyl acetate to yield a light yellow solid (0.250 g, 87%). mp. 146-148 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.40 (s, 4H), 4.35 (q, J = 7.1 Hz, 4H), 1.37 (t, J = 7.1 Hz, 6H) **(Figure A.13, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 160.7, 144.9, 111.7, 64.7, 61.2, 14.2 **(Figure A.14, Appendix A)**. IR (ATR, cm^{-1}): 2995, 2938 (-CH st), 1675 (C=O st), 1084 (-C-O st) **(Figure A.15, Appendix A)**. MS: $[M+H]^+$ m/z = 287.21 **(Figure A.16, Appendix A)** [37].

2.3.6 Diethyl 2-(hydroxymethyl)-2,3-dihydrothieno[3,4-b]-1,4-dioxine-5,7-dicarboxylate 3c



3c

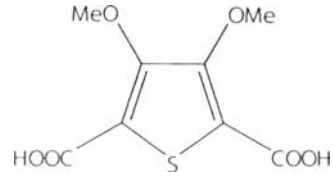
Compound DDTD (0.260 g, 1.0 mmol), epichlorohydrin (0.47 mL, 6.0 mmol) and K_2CO_3 (0.28 g, 2.0 mmol) were mixed in EtOH (20 mL). The reaction was stirred and refluxed at 80 °C for 72 h under nitrogen atmosphere. The reaction mixture was quenched by 10% hydrochloric acid solution and then extracted twice with chloroform. The combined organic layers were washed by 2 M NaOH, dried over anhydrous $MgSO_4$, and evaporated using rotary evaporator to get yellow solid. The crude mixture was purified by passing through a silica gel column, eluted with hexane:ethyl acetate (2:1) to yield a light yellow solid (0.180 g, 57%). ^{1}H NMR (400

MHz, $CDCl_3$): δ (ppm) 4.47 (m, 2H), 4.36 (m, 1H), 4.27 (m, 2H), 3.94 (q, *J* = 12.5, 4H), 1.36 (s, 1H), 1.33 (t, 6H) **(Figure A.17, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 161.0, 160.8, 145.3, 144.7, 112.0, 111.3, 74.8, 74.5, 66.0, 61.4, 60.9, 14.2, 14.2 **(Figure A.18, Appendix A)**. IR (ATR, cm^{-1}): 3539 (-OH st), 2987, 2934 (-CH st), 1702 (C=O st) **(Figure A.19, Appendix A)** [38].

2.3.7 3,4-Dialkoxythiophene-2,5-dicarboxylic acid 4

General procedure: One equivalent of diethyl 3,4-dialkoxythiophene-2,5-dicarboxylate **3** was stirred and refluxed at 80 °C for 10 h under nitrogen with 1 M NaOH and EtOH. The reaction solution was cooled to room temperature and acidified by concentrated hydrochloric acid. The product was filtered, washed with water, and dried to obtain the relatively pure corresponding white solid product [36].

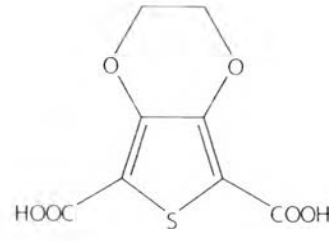
2.3.7.1 3,4-Dimethoxythiophene-2,5-dicarboxylic acid 4a



4a

Following the general procedure, diethyl 3,4-dimethoxythiophene-2,5-dicarboxylate **3a** (0.280 g, 1.0 mmol); 10 mL of 1 M NaOH and 1 mL EtOH were used. The precipitate was collected by filtration and washed with water to obtain diacid **4a** as a white solid (0.178 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 3.88 (s, 6H) **(Figure A.20, Appendix A)**. ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 161.4, 153.3, 119.6, 61.6 **(Figure A.21, Appendix A)**. IR (ATR, cm^{-1}): 3079 (-OH st), 2925 (-CH st), 1724 (C=O st), 1189 (-C-O st) **(Figure A.22, Appendix A)** [36].

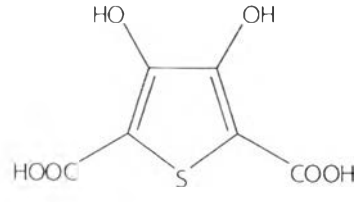
2.3.7.2 2,3-Dihydrothieno[3,4-b]-1,4-dioxine-5,7-dicarboxylic acid 4b



4b

Following the general procedure, diethyl 2,3-dihydrothieno[3,4-*b*]-1,4-dioxine-5,7-dicarboxylate **3b** (0.280 g, 1.0 mmol): 10 mL of 1 M NaOH and 1 mL EtOH were used. The precipitate was collected by filtration and washed with water to obtain diacid **4b** as a white solid (0.220 g, 96 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 4.32 (s, 4H) **(Figure A.23, Appendix A)**. ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 161.7, 144.8, 111.6, 64.2 **(Figure A.24, Appendix A)**. IR (ATR, cm^{-1}): 3553 (-OH st), 2938 (-CH st), 1652 (C=O st), 1079 (-C-O st) **(Figure A.25, Appendix A)**.

2.3.7.3 3,4-Dihydroxy-2,5-dicarboxylic acid 4d



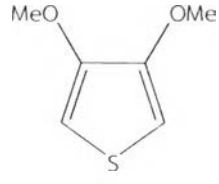
4d

Following the general procedure, Compound DDTD (0.260 g, 1.0 mmol); 10 mL of 1 M NaOH and 1 mL EtOH were used. The aqueous layer was separated and evaporated. The obtained solid was then recrystallized from methanol to obtain diacid **4d** as a light gray solid (0.220 g, 98 %). ^{1}H-NMR (400 MHz, DMSO-d_6): δ (ppm). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 171.2, 170.6, 76.1 **(Figure A.26, Appendix A)**. IR (ATR, cm^{-1}): 3305 (-OH st), 1663 (C=O st) **(Figure A.27, Appendix A)**. MS: $[M+H]^+$ m/z = 204.99 **(Figure A.28, Appendix A)**.

2.3.8 3,4-Dialkoxythiophenes 5

General procedure: One equivalent of 3,4-dialkoxythiophene-2,5-dicarboxylic acid **4** was stirred and refluxed at 150 °C with 17 mol % of cuprous oxide (Cu_2O) in quinoline for 20 h under nitrogen atmosphere. After cooling, the mixture was filtered and poured into an excess of 10% hydrochloric acid solution. The product was extracted with ethyl acetate, washed with 10% hydrochloric acid and water. The solvent was removed under reduced pressure after drying the organic layer over anhydrous $MgSO_4$. The product was purified by column chromatography using hexane:ethyl acetate as eluent to give the corresponding product [36].

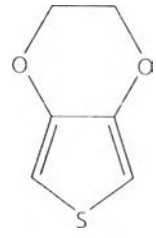
2.3.8.1 3,4-Dimethoxythiophene 5a



5a

Following the general procedure; 3,4-dimethoxythiophene-2,5-dicarboxylic acid **4a** (0.230 g, 1.0 mmol); Cu_2O (0.025 g, 0.17 mmol); 10 mL of quinoline were used. Column chromatography purification of the product using hexane:ethyl acetate (2:3) as eluent afforded 3,4-dimethoxythiophene **5a** as yellow oil (0.089 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.20 (s, 1H), 3.87 (s, 3H) **(Figure A.29, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 147.9, 96.3, 57.6 **(Figure A.30, Appendix A)**. IR (ATR, cm^{-1}): 2985 (-CH st), 1735 (C=C), 1045 (C-O st) **(Figure A.31, Appendix A)** [34, 36].

2.3.8.2 2,3-Dihydrothieno[3,4-b]-1,4-dioxine (EDOT, 5b)



5b

Following the general procedure; 2,3-dihydrothieno[3,4-*b*]-1,4-dioxine-5,7-dicarboxylic acid **4b** (0.230 g, 1.0 mmol); Cu_2O (0.025 g, 0.17 mmol); 10 mL of quinoline were used. Column chromatography purification of the product using hexane:ethyl acetate (1:3) as eluent afforded 2,3-dihydrothieno[3,4-*b*]-1,4-dioxine (EDOT, **5b**) as yellow oil (0.100 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.32 (s, 1H), 4.20 (s, 2H) **(Figure A.32, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 138.7, 98.3, 64.6 **(Figure A.33, Appendix A)**. IR (ATR, cm^{-1}): 3113 (-CH_a st), 2984 (-CH st), 1490 (C=C st), 1056 (CH_2O st) **(Figure A.34, Appendix A)** [39].

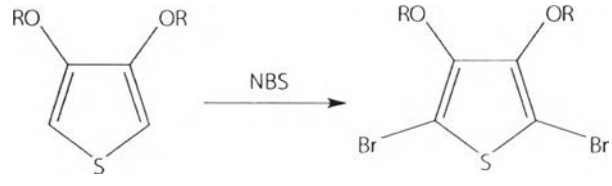
2.3.9 2,3-Dihydrothieno[3,4-*b*]-1,4-dioxin-2-yl methanol (EDTM, 6)

6

3,4-dimethoxythiophene **5a** (0.144 g, 1.0 mmol), glycerol (0.552 g, 6.0 mmol) and *p*-toluenesulfonic acid (PTSA) (0.038 g, 0.2 mmol) was stirred in 15 mL toluene and refluxed at 110 °C for 48 h under nitrogen atmosphere. After completion, the reaction was quenched by adding saturated $NaHCO_3$ solution. Then the organic layer was separated and the aqueous layer was extracted with ethyl acetate three times. The combined organic layers were dried over anhydrous $MgSO_4$. The crude mixture was purified by column chromatography using hexane:ethyl acetate (1:1) as eluent to

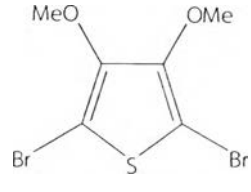
yield 2,3-dihydrothieno[3,4-*b*]-1,4-dioxin-2-yl methanol (EDTM, **6**) as yellow oil (0.070 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.27 (s, 2H), 4.15 (m, 2H), 4.02 (m, 1H), 3.77 (m, 2H), 1.94 (s, 1H) **(Figure A.35, Appendix A)**. ^{13}C NMR δ ($CDCl_3$) (ppm): 141.4, 141.4, 100.2, 99.8, 74.1, 65.7, 61.6 **(Figure A.36, Appendix A)**. IR (ATR, cm^{-1}): 3386 (-OH st), 3114 (-CH st), 2923, 1485 (C=C st), 1183 (-C-O st) **(Figure A.37, Appendix A)** [38].

2.3.10 Brominations of thiophene derivatives 7



General procedure: 2.5 equivalents of *N*-bromosuccinimide (NBS) were added to a stirred solution of thiophene precursor (1.0 mmol) in dichloromethane (10 mL) at room temperature. After completion, the reaction mixture was quenched by adding saturated $NaHCO_3$ solution. The organic layer was separated and the aqueous layer was extracted with dichloromethane three times. Then the combined organic layers were washed with 2 M NaOH three times. After drying over anhydrous $MgSO_4$, the solution was evaporated using rotary evaporator and then purified by column chromatography to obtain the corresponding dibromothiophene [40].

2.3.10.1 2,5-Dibromo-3,4-dimethoxythiophene (DBDMT, 7a)

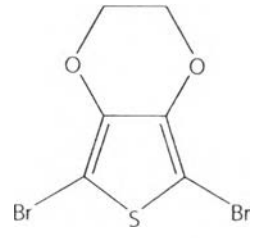


7a

Following the general procedure; 3,4-Dimethoxythiophene **5a** (0.144 g, 1.0 mmol) and NBS (0.4450 g, 2.5 mmol) in dichloromethane (10 mL) were mixed for 10 min. The crude mixture was purified by column chromatography, eluted with 3:1 mixture of hexane and ethyl acetate to get yellow oil of product (0.240 g, 82%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.91 (s, 6H) **(Figure A.38, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 149.0, 94.8, 60.9 **(Figure A.39, Appendix A)**. IR (ATR, cm^{-1}): 2945 (-CH st), 1730 (C=C st), 1045 (-C-O st) **(Figure A.40, Appendix A)**. MS: $[M+H]^+$ m/z = 301.88 **(Figure A.41, Appendix A)**.

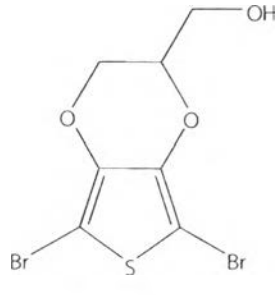
2.3.10.2 2,5-Dibromo-3,4-ethylenedioxythiophene (DBEDOT, 7b)



7b

Following the general procedure; 3,4-Ethylenedioxythiophene (EDOT, **5b**) (0.142 g, 1.0 mmol) and NBS (0.4450 g, 2.5 mmol) in chloroform (10 mL) were mixed for 2 min. The crude mixture was purified by column chromatography, eluted with 3:2 mixture of hexane and ethyl acetate to get a light yellow solid (0.290 g, 98%). mp. 96-97 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.27 (s, 4H) **(Figure A.42, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 139.7, 85.5, 64.9 **(Figure A.43, Appendix A)**. IR (ATR, cm^{-1}): 2923 (-CH st), 1505 (C=O st), 1080 (-C-O st) **(Figure A.44, Appendix A)**. MS: $[M+H]^+$ m/z = 299.20 **(Figure A.45, Appendix A)** [41].

2.3.10.3 2,5-Dibromo[3,4-b]-1,4-dioxin-2-yl methanol (DBEDTM, 7c)

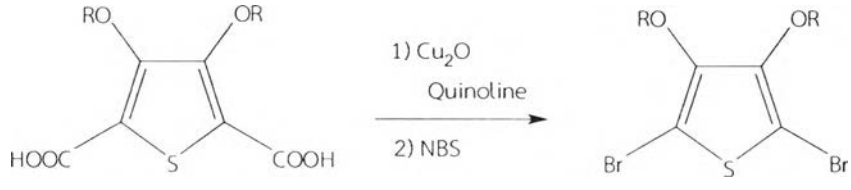


7c

Following the general procedure; 2,3-Dihydrothieno[3,4-*b*]-1,4-dioxin-2-yl methanol (EDTM, **6**) (0.172 g, 1.0 mmol) and NBS (0.4450 g, 2.5 mmol) in chloroform

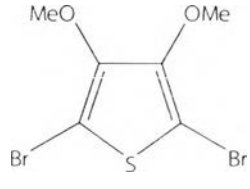
(10 mL) were mixed for10 min. The crude mixture was purified by column chromatography, eluted with ethyl acetate to get a pale yellow liquid (0.250 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.24 (m, 2H), 4.10 (m, 1H), 3.84 (m, 2H), 1.99 (s, 1H) **(Figure A.46, Appendix A)**. ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 139.5, 139.5, 85.6, 85.5, 74.6 65.1, 61.4 **(Figure A.47, Appendix A)**. MS: $[M+H]^+$ m/z = 329.09 **(Figure A.48, Appendix A)**.

2.3.11 Bromodecarboxylation of 3,4-dialkoxythiophene-2,5-dicarboxylic acid (4)



General procedure: One equivalent of 3,4-dialkoxythiophene-2,5-dicarboxylic acid was heated at 150 °C with 17 mol % of cuprous oxide (Cu_2O) in 10 mL quinoline for 20 h under nitrogen. After cooling, 2.5 equivalents of *N*-bromosuccinimide (NBS) in 10 mL of dichloromethane were added into the reaction mixture and stirred at room temperature. After completion, the reaction mixture was quenched by adding saturated $NaHCO_3$ solution and then poured into an excess of 10% hydrochloric acid solution. The product was extracted with ethyl acetate and washed with 2 M NaOH three times. Ethyl acetate was removed under reduced pressure after drying the organic layer over anhydrous $MgSO_4$. The products were purified by column chromatography using hexane:ethyl acetate as eluent [36, 40].

2.3.11.1 2,5-Dibromo-3,4-dimethoxythiophene (DBDMT, 7a)

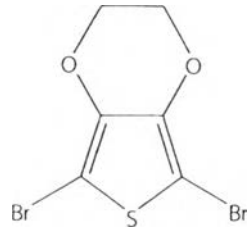


7a

Following the general procedure; 3,4-dimethoxythiophene-2,5-dicarboxylic acid **4a** (0.230 g, 1.0 mmol); Cu_2O (0.025 g, 0.17 mmol) in 10 mL of quinoline were

used followed by reaction with NBS (0.4450 g, 2.5 mmol) for 10 min. The crude mixture was purified by passing through a silica gel column, eluted with 6:1 mixture of hexane and ethyl acetate to get light yellow oil product (0.091 g, 29%).

2.3.11.2 2,5-Dibromo-3,4-ethylenedioxythiophene (DBEDOT, 7b)

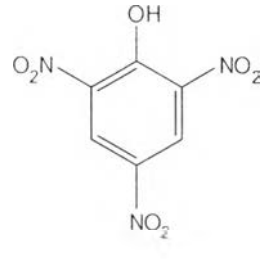


7b

Following the general procedure; 2,3-dihydrothieno[3,4-*b*]-1,4-dioxine-5,7-dicarboxylic acid **4b** (0.230 g, 1.0 mmol); Cu_2O (0.025 g, 0.17 mmol) in 10 mL of quinoline were used followed by reaction with NBS (0.4450 g, 2.5 mmol) for 2 min. The crude mixture was purified by passing through a silica gel column, eluted with 1:1 mixture of hexane and ethyl acetate to get a light yellow solid product (0.230 g, 77%).

2.4 Preparations of template molecules

2.4.1 2,4,6-Trinitrophenol (TNP)

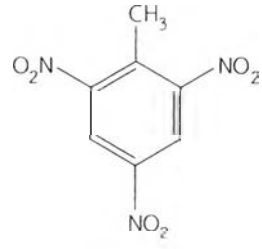


TNP

p-Nitrophenol (1.000 g, 0.007 mol) was added into concentrated nitric acid (8 mL) at -5 °C in ice-salt bath and stirred the mixture for 30 min. The reaction was allowed to warm and stir at room temperature for 5 h. It was quenched by adding 10 mL saturated $NaHCO_3$ solution. The obtained yellow precipitate was filtered and washed with water to obtain a yellow solid product (1.097 g, 67%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.21 (s, 2H), 11.91 (s, 1H) **(Figure A.49, Appendix A)** [42].

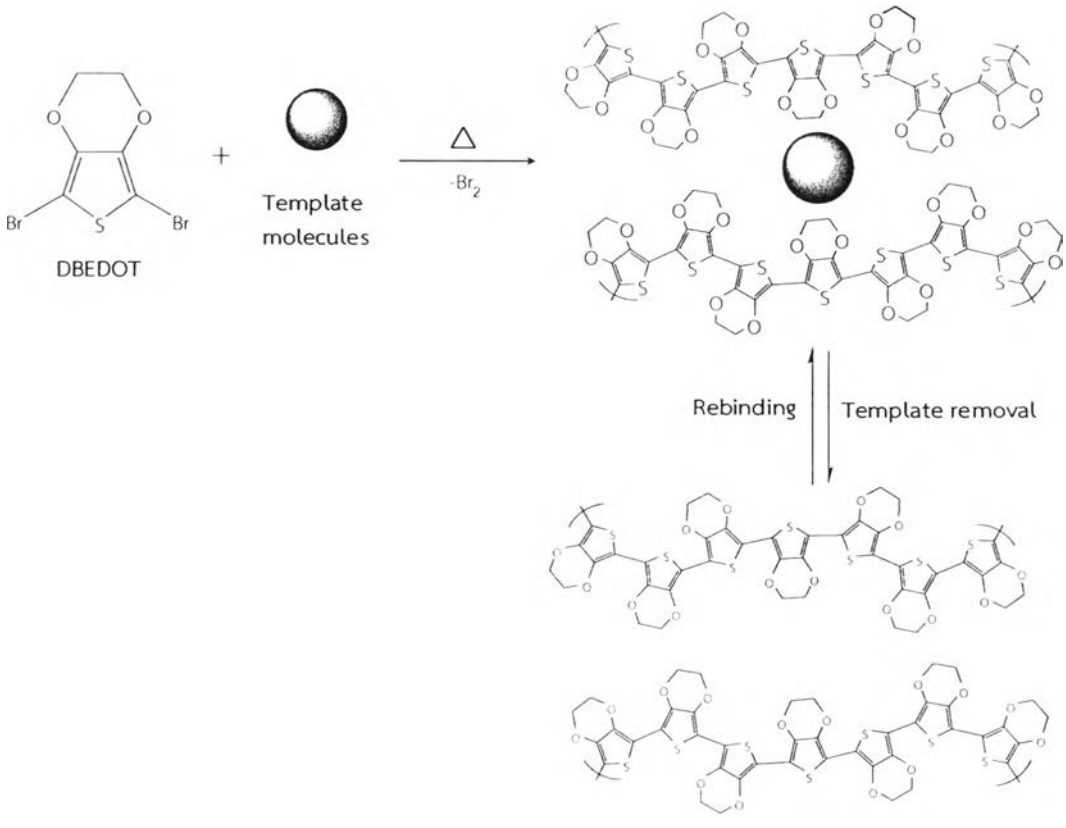
2.4.2 2,4,6-Trinitrotoluene (TNT)



TNT

Toluene (1.000 g, 0.011 mol) was added to the mixture of fuming nitric acid (15 mL) and concentrated sulfuric acid (10 mL). The reaction was stirred for 3 h at room temperature and another 5 h at 85 °C. After cooling back to room temperature, the reaction was quenched by adding 10 mL saturated $NaHCO_3$ solution. The precipitate was collected by filtration and washed with water to obtain as a light yellow solid product (1.830 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.85 (s, 2H), 2.72 (s, 3H) **(Figure A.50, Appendix A)** [43].

2.5 Preparation of molecularly imprinted polymers (MIPs)



1000 ppm (0.010 g, 0.043 mmol) of 2,4,6-trinitrophenol (TNP) was added into 20 mL ethyl acetate solution of 2,5-dibromo-3,4-ethylenedioxythiophene (DBEDOT) (0.299 g, 1.0 mmol). The solvent was removed from the homogeneous mixture using rotary evaporator. The obtained solid was heated in an oven at 85 °C for 72 h. During this period the solid turned dark blue with slight appearance of brown bromine vapor. The resulting dark blue solid was then allowed to cool to room temperature as the 2,4,6-trinitrophenol-molecularly imprinted polymers (TNP-MIPs). The process was similarly repeated with 2,4,6-trinitrotoluene (TNT) (0.010 g, 0.044 mmol) as the template, yielding the 2,4,6-trinitrotoluene-molecularly imprinted polymers (TNT-MIPs). As a control, the non-molecularly imprinted polymers (NIPs) were prepared similarly in the absence of the template molecules.

After the polymerization, all MIPs and NIPs samples were exhaustive extraction by Soxhlet extraction with methanol for 16 h or until no template molecules or leftover monomer was detected by gas chromatography. The vacant polymer samples were kept dry in desiccator overnight.

2.6 Binding experiments

Five exact concentrations of the template molecule solutions were prepared and measured their UV-Visible absorption to obtain a calibration curve. Then 25 mL of 1000 ppm of the template solution was added to each of its corresponding MIP and NIP samples. The mixtures were stirred at room temperature. 1 mL of the liquid were drawn from each solution every 1 hour and diluted to an appropriate concentration before being measured the absorbance until the value became constant where the equilibrium was reached. The amount of the binding capacities of the MIP was also examined by extracting off the bound template molecules from the equilibrated MIP and NIP samples by another Soxhlet extraction with methanol for 8 h.

The amount of template molecules bound to the polymers (Q) was calculated by subtracting the amount of unbound substrate from its initial concentration. To compare the imprinting effect, we defined the specific adsorption values as $\Delta Q = Q_{MIPs}-Q_{NIPs}$, where Q_{MIPs} and Q_{NIPs} are the amounts of bound template molecules on the imprinted and non-imprinted polymers at equilibrium, respectively. The average of triplicate independent experiments was used for the analysis and discussion [44, 45].

The binding process was monitored by UV-Vis spectroscopy, measuring at the λ_{max} of 336 nm and 255 nm for TNP and TNT, respectively. Calibration curve of template was prepared and measured by plotting a graph between absorbance and concentrations of template solution (0.88—4.35 μmol/mL for TNP template solution and 0.83—4.30 μmol/mL for TNT template solution) **(Figure B.1 and Figure B.2, Appendix B)**, a linear relationship was obtained and used for calculations of ΔQ, Q_{MIPs}, and Q_{NIPs} values.

2.7 Rebinding experiments

After Binding experiments, all the MIPs based on poly(3,4-ethylene-dioxythiophene) (PEDOT) and NIPs samples were cleaned up extracted by exhaustive Soxhlet extraction with methanol for 8 h or until the template molecules was not detected by gas chromatography. After being dried, the binding experiments were repeated on these samples using the same procedure as in 2.6. The specific adsorption values (ΔQ) were calculated to prove the reusability of the MIPs.