CHAPTER III

EXPERIMENTAL

3.1 Chemicals

All chemicals were purchased from commercial source and used as received, unless noted otherwise.

- Sodium metal (Na) : Merck
- Ethanol (CH_3CH_2OH) : Merck
- Diethyl oxalate : Merck
- Acetone : Merck
- Hydrochloric acid (HCl) : Merck
- Ammonia (25% NH_3) : Merck
- Charcoal : Laboratory Rasayan
- Phenylphosphonic dichloride : Sigma-Aldrich
- Sodium hydrogencarbonate ($NaHCO_3$) : Carlo Erba
- Sodium chloride (NaCl) : Merck
- Sodium sulfate (Na_2SO_4) : Merck
- Hexanes : Distelled from commercialgrade
- Ethylacetate (EtOAc) : Distelled from commercial grade
- Methylene chloride (CH_2Cl_2) : Distelled from commercial grade
- Potassium carbonate (K_2CO_3) : Carlo Erba
- Sodium hydride (NaH) : Sigma-Aldrich
- Diethyl malonate : Sigma-Aldrich
- Toluene : Lab-Scan

- Thionyl chloride ($SOCl_2$) : Merck
- Sodium hydroxide (NaOH) : Merck
- Sodium ethoxide (NaOEt) : Sigma-Aldrich
- Potassium hydroxide (KOH) : Carlo Erba
- 2-Amino-3-pyridinecarboxaldehyde : Sigma-Aldrich
- Diethyl ether : Merck
- Potassium dichromate ($K_2Cr_2O_7$) : Merck
- Sulfuric acid (H_2SO_4) : Merck
- Ruthenium(III) chloride hydrates ($RuCl_3 \cdot nH_2O$) : Sigma-Aldrich
- Deuterated chloroform ($CDCl_3$) : Merck
- Deuterated dimethyl sulfoxide-d_6 (DMSO- d_6) : Merck
- Silica gel : Merck

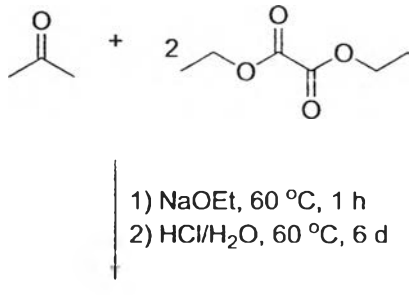
3.2 Analytical instruments

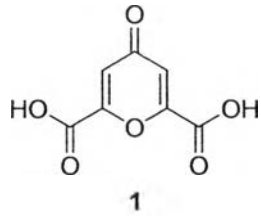
^{1}H-NMR were recorded on Varian (300 or 400 MHz) and ^{13}C-NMR were recorded on Inova and Bruker (100 MHz) Ultrashiel. Chemical shift (δ) were reported in parts per million (ppm) relative to the residual $CHCl_3$ or DMSO peak (7.26 or 2.50 ppm, respectively, for ^{1}H-NMR and 77.0 or 39.43 ppm, respectively, for ^{13}C-NMR). Coupling constant (J) are reported in Hertz (Hz). Mass spectra were obtained from high-resolution electrospray ionization mass spectrometry (HR ESI-MS), electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted laser desorption ionization (MALDI) mass spectrometry with dithranol as a matrix. UV-visible absorption spectra were recorded in DMSO by a Hewlett-Packard 8453 spectrophotometer and

absorption extinction coefficients (ε) were recorded in L/mol·cm. FT-IR spectra were recorded between 400 cm^{-1} to 4,000 cm^{-1} in transmittance mode on a Fourier Transform Infrared Spectrophotometer: Impact 410 (Nicolet Instruments Technologies, Inc, WI, USA). Fluorescence spectra were measured in DMSO using a Perkin-Elmer LS45 luminescence spectrophotometer. Absorption and emission spectra of the solutions were measured in DMSO at room temperature.

3.3 Experimental procedures

3.3.1 4-Oxo-4H-pyran-2,6-dicarboxylic acid (chelidonic acid) (1)



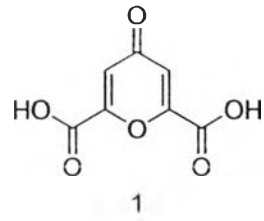


1

Following a literature method [29], sodium metal (20.00 g, 0.870 mol) was dissolved in ethanol (220 mL) under nitrogen. This sodium ethoxide solution was added to a solution of acetone (16.0 mL, 0.217 mol) and diethyl oxalate (73.6 mL, 0.540 mol) in round-bottomed flask. The mixture was refluxed at 60 °C for 1 h. After that, a 37% aqueous HCl (40 mL) and water (20 mL) were added. A brown precipitate was then formed. After the solvent was evaporated out, water (70 mL) and a 37% aqueous HCl (10 mL) were added to the resulting brown crude product. The mixture was refluxed at 60 °C for 6 d. The precipitate was collected by filtration and

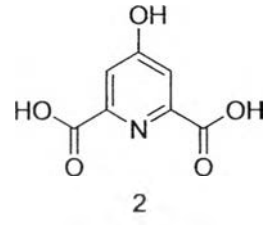
dissolved in the boiled water. After the precipitate was completely dissolved, charcoal was added and the solution was filtered. The compound **1** was obtained as a white crystal after cooled down at room temperature. (32.6 g, 81%), ^{1}H-NMR (400 MHz, DMSO-d_6): δ 6.86 (s, 2H) ppm (**Figure A.1**); ^{13}C-NMR (DMSO-d_6): δ 117.7, 155.8, 160.8, 179.7 ppm (**Figure A.2**). Other spectroscopic data are consistent with those described in the literature.

3.3.2 4-Oxo-1,4-dihydropyridine-2,6-dicarboxylic acid (Chelidamic acid) (2)



1

25% aq. NH_3, rt , 2 d

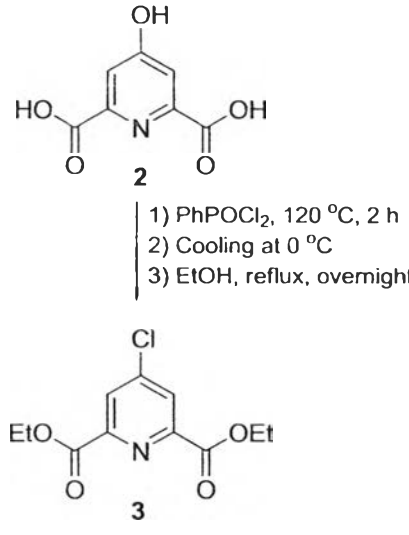


2

Following a literature method [29], a 25% aqueous NH_3 solution (250 mL) was added to compound **1** (8.00 g, 0.044 mol) at 0 °C. The mixture was stirred at room temperature for 2 d (the mixture became an orange solution after five h). The solvent was evaporated out. The brown crude was obtained and boiled with water in the presence of charcoal for 5 min. After the filtration, the solution was cooled at 0 °C for 15 min. The solution was acidified with a 37% aqueous HCl solution to pH 1. The white precipitate was formed and collected by filtration. The white precipitate was dried under vacuum for 3 h to give compound **2** as a white solid (6.6 g , 82%).

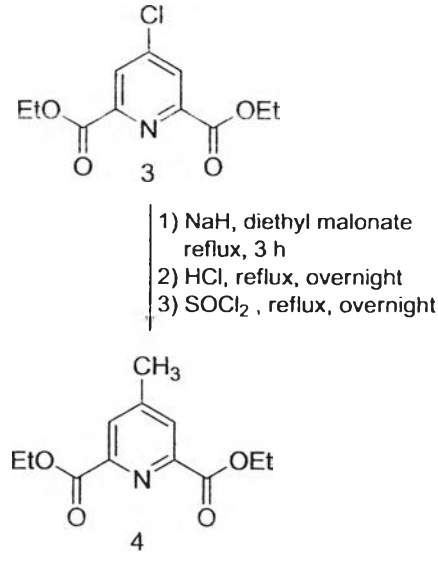
^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.56 (s, 2 H) ppm (**Figure A.3**) ; ^{13}C-NMR (DMSO-d_6): δ 114.2, 148.7, 165.1, 165.9 ppm (**Figure A.4**). Other spectroscopic data are consistent with those described in the literature.

3.3.3 4-Chloropyridine-2,6-dicarboxylate (3)



Following a published procedure [30], phenylphosphonic dichloride (22.0 mL, 0.109 mol) was added to chelidamic acid (5.00 g, 0.027 mol) . The mixture was heated at 120 °C for 2 h. After cooling at 0 °C, the mixture was treated with an excess amount of ethanol (30.0 mL, 0.513 mol) and refluxed overnight. The solvent was evaporated out. The brown crude product was dissolved by CH_2Cl_2 and washed with 4% aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated out and the resulting brown oil product was purified by a silica column (hexane:EtOAc, 3:1) to give diethyl 4-chloropyridine-2,6-dicarboxylate (**3**) as a white crystal (3.47 g, 50%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 2H), 4.50 (q, J = 7.2 Hz, 4H), 1.38 (t, J = 7.2 Hz, 6H) ppm (**Figure A.5**); ^{13}C-NMR ($CDCl_3$): δ 14.2, 62.7, 128.0, 146.6, 149.9, 163.6 ppm (**Figure A.6**). Other spectroscopic data are consistent with those described in the literature.

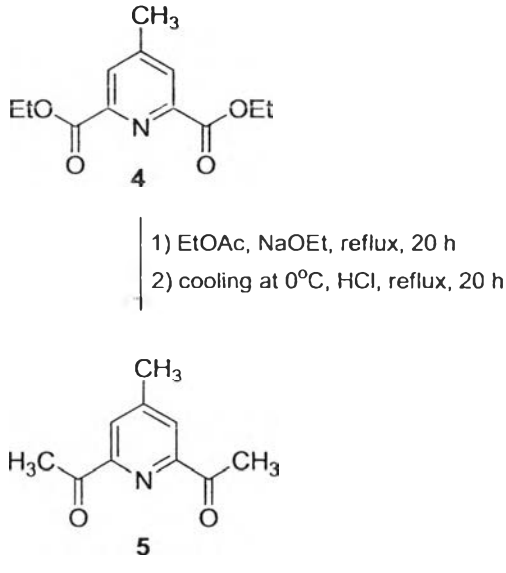
3.3.4 Diethyl 4-methylpyridine-2,6-dicarboxylate (4)



Diethyl 4-methylpyridine-2,6-dicarboxylate (**4**) was prepared by a method described in a literature [31]. A suspension of sodium hydride (0.78 g, 0.033 mol, 60% oil dispersion) in toluene (20 mL), was carefully treated with diethyl malonate (0.50 g, 0.033 mmol) under nitrogen. The resulting malonate salt was formed and completely dissolved upon heating at reflux. Diethyl 4-chloropyridne-2,6-dicarboxylate (**3**) (1.49 g, 0.058 mol) was added and then refluxed for 3 h. The pasty oil formed at the bottom of the flask. The hot solution was removed out by decantation and a 4M HCl solution (20 mL) was added to the residual oil. The mixture was refluxed overnight. After cooling, the aqueous phase was washed with Et_2O. The aqueous phases was evaporated out. The resulting brown powder product was dissolved in 1M NaOH (10 mL) and then 2M HCl was added. The precipitate was filtered off and dried at 120 $^{\circ}C$ for 3 h to give a brown solid (1.82 g). In a 50 mL round bottomed flask equipped with a condenser, the brown solid was added into a mixture of $SOCl_2$ (2 mL) and EtOH (20 mL) and refluxed overnight. After cooling, insoluble material was filtered off. The resulting filtrate was concentrated to dryness to give a brown. The brown crude was dissolved by CH_2Cl_2 and washed with a

saturated solution of $NaHCO_3$ and brine. Then organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated out, leading to the brown oil product that was purified by crystallization from hexanes to give diethyl 4-methylpyridine-2,6-dicarboxylate (**4**) as a white crystal (0.48 g, 44%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 2H), 4.44 (q, J = 7.2 Hz, 4H), 2.48 (s, 3H), 1.42 (t, J = 7.2 Hz, 6H) ppm. (**Figure A.7**). Other spectroscopic data are consistent with those described in the literature.

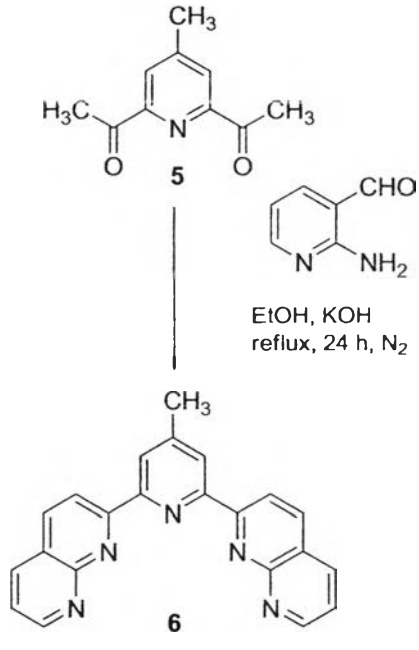
3.3.5 1,1'-(4-Methylpyridine-2,6-diyl)diethanone (5)



Following a previously published method [32] with a slight modification, diethyl 4-methylpyridine-2,6-dicarboxylate (**4**) (0.400 g, 1.69 mmol) was added in freshly distilled EtOAc (10 mL). Sodium ethoxide (0.580 g, 8.45 mmol) was then added and refluxed for 20 h. After cooling at 0 $^{\circ}$C, the mixture was added with an excess amount of concentrated HCl (4 mL) and then refluxed for additional 20 h. A white solid (NaCl) was precipitated. After cooling, the mixture was added with water for dissolve the NaCl formed. The mixture was washed with 5% aqueous Na_2CO_3 and the aqueous phase was extracted by EtOAc. Then organic layer was dried over

anhydrous Na_2SO_4 and filtered off. The solvent was evaporated out. The brown crude product was purified by a silica column (hexane:EtOAc, 4:1) to give 1,1'-(4-methylpyridine-2,6-diyl)diethanone (**5**) as a white crystal (0.05 g, 17%). m.p. 100.5–102.0 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.49 (s, 3H), 2.78 (s, 6H), 8.03 (s, 2H) ppm (**Figure A.8**) ; ^{13}C-NMR ($CDCl_3$): δ 21.1, 25.6, 125.4, 149.5, 152.7, 199.7 ppm (**Figure A.9**); HR-ESI-MS m/z obsd. 200.0691, calcd. 200.0687 ($[M + Na]^+$; M = $C_{10}H_{11}NO_2$) (**Figure A.10**).

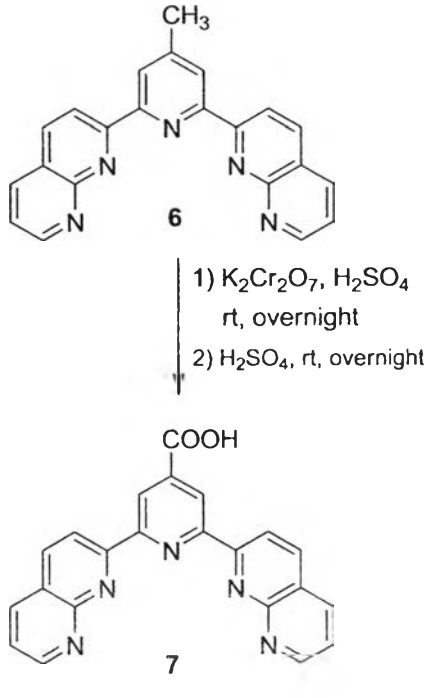
3.3.6 2,2'-(4-Methylpyridine-2,6-diyl-bis-naphthyridine) (6)



Following a published procedure [26] with a slight modification, a solution of 1,1'-(4-methylpyridine-2,6-diyl)diethanone (**5**) (50.0 mg, 0.282 mmol) and 2-aminonicotinaldehyde (70.0 mg, 0.564 mmol) in ethanol (5 mL) was treated with a saturated solution of potassium hydroxide in ethanol (0.28 mL) and refluxed for 24 h under nitrogen atmosphere. After cooling down to room temperature, the mixture was filtered and the resulting solid was further purified by crystallization ($CHCl_3$) to

give a light yellow solid (0.07 g, 68%). m.p. > 300 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.61 (s, 3H), 7.53 (dd, *J* = 4.0, 8.0 Hz, 2H), 8.27 (dd, *J* = 1.6, 8.0 Hz, 2H), 8.38 (d, *J* = 8.4 Hz, 2H) 8.88 (s, 2H), 9.00 (d, *J* = 8.4 Hz, 2H), 9.18 (dd, *J* = 1.6, 4.0 Hz, 2H) ppm (**Figure A.11**); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.65 (s, 3H), 7.68 (dd, *J* = 4.0, 8.0 Hz, 2H), 8.56 (dd, *J* = 1.6, 8.0 Hz, 2H), 8.63 (s, 2H), 8.69 (d, *J* = 8.4 Hz, 2H), 8.99 (d, *J* = 8.4 Hz, 2H), 9.15 (dd, *J* = 1.6, 4.0 Hz, 2H) ppm (**Figure A.12**); ^{13}C-NMR ($CDCl_3$): δ 21.2, 120.3, 122.1, 122.9, 124.4, 136.9, 137.7, 149.7, 153.8, 154.6, 155.9, 159.4 ppm (**Figure A.13**); Due to low solubility of **6** in DMSO-d_6, its ^{13}C-NMR spectrum in DMSO-d_6 was not well resolved; ESI-HR-MS m/z obsd. 372.1239, calcd. 372.1225 ($[M + Na]^+$; M = $C_{22}H_{15}N_5$) (**Figure A.14**); IR υ (cm^{-1}) 1,597 (C=N stretching), 1,549 (C=C stretching), 2,925 (C–H stretching) (**Figure A.15**); λ_{abs} (ε) 335, 337 (3.6×10^4) nm (**Figure B.1**) ; λ_{em} (λ_{ex} = 350 nm) 385, 440 nm (**Figure B.3**).

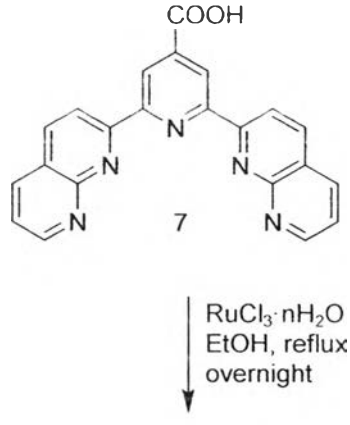
3.3.7 2,6-Di(1,8-naphthyridine-2-yl)isonicotinic acid (7)

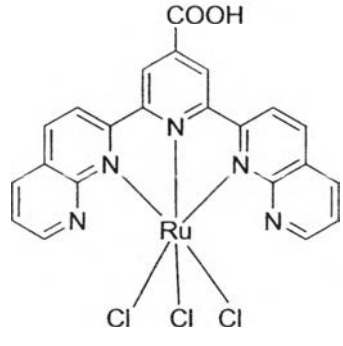


Following a previously reported method [6] with a slight modification, a mixture of 2,2'-(4-methylpyridine-2,6-diyl)-bis-(1,8-naphthyridine) (**6**) (0.15 g, 0.43 mmol) in concentrated H_2SO_4 (3 mL) was treated with $K_2Cr_2O_7$ (0.29 g, 0.98 mmol) in an ice bath over a period of 1 h. A mixture was stirred at room temperature overnight. The deep green mixture was poured into iced water (70 mL) and adjusted to pH 3 with Na_2CO_3. The precipitate was filtered off, washed with water and dried to give a green solid.

Following a literature method [33], a mixture of the green solid in concentrated H_2SO_4 (15 ml) was stirred at room temperature overnight. The resulting greenish-yellow solution was poured into an ice-water mixture (100 mL). The precipitate was filtered off, washed with water and dried to give compound **7** as a brown solid (0.06 g, 70%). m.p. > 300 ^{o}C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.73 (dd, J = 4.5, 8.1 Hz, 2H), 8.61 (dd, J = 2.1, 8.1 Hz, 2H), 8.74 (d, J = 8.4 Hz, 2H), 9.00 (d, J = 8.4 Hz, 2H), 9.22 (m, 4H) ppm (**Figure A.16**); ^{13}C-NMR (DMSO-d_6): δ 119.7, 121.7, 122.2, 138.0, 139.4, 154.2, 154.8, 155.8, 157.2, 165.8 ppm (**Figure A.17**); ESI-HR-MS m/z obsd. 402.0971, calcd. 402.0967 ($[M + Na]^+$; M = $C_{22}H_{13}N_5O_2$) (**Figure A.18**), IR υ (cm^{-1}) 1,602 (C=N stretching), 1,553 (C=C stretching), 1,713 (C=O stretching), 3,067 (C–H stretching), 3,200–3,500 (O–H stretching) (**Figure A.19**); λ_{abs} (ε) 326, 340 (2.9×10^4) nm (**Figure B.4**) ; λ_{em} (λ_{ex} = 350 nm) 389, 435 nm (**Figure B.6**).

3.3.8 Ruthenium complex of 2,6-di(1,8-naphthyridine-yl)isonicotinic acid (8)





8

Following a standard method [10] with a slight modification, a mixture of 2,6-di(1,8-naphthyridine-2-yl)isonicotinic acid (**7**) (60.0 mg, 0.145 mmol) and $RuCl_3 \cdot nH_2O$ (30.0 mg, 0.145 mmol.) in ethanol (3 mL) was refluxed overnight. A resulting dark green precipitate was filtered off, washed with water and dried to give compound **8** as a dark green solid (0.05 g, 61%). m.p. > 300 °C. Due to low solubility of **8**, NMR measurement could not be performed. ESI-HR-MS m/z obsd. 549.9458, calcd. 549.9412 ($[M-H-Cl]^+$; M = $C_{22}H_{12}Cl_2N_5O_2Ru$) (**Figure A.20**); IR υ (cm^{-1}) 1,603 (C=N stretching), 1,557 (C=C stretching), 1,721 (C=O stretching), 3,062 (C–H stretching), 3,200–3,500 (O–H stretching) (**Figure A.21**); λ_{abs} (ε) 337, 340 (2.9×10^4) nm (**Figure B.7**) ; λ_{em} (λ_{ex} = 350 nm) 417, 465 nm (**Figure B.9**).