CHAPTER II

EXPERIMENTAL

2.1 Materials and chemicals

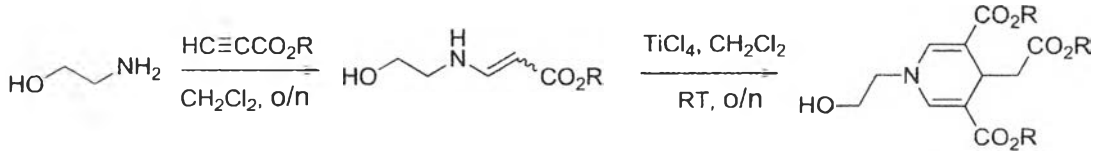
Ethanolamine, ethyl propiolate, tosyl chloride (TsCl), titanium tetrachloride ($TiCl_4$), triethylamine (TEA), diethylamine (DEA) and azacrown ether (n=1-3) were purchased from Sigma-Aldrich and Fluka. DHP-OH was prepared by our previously reported procedure [12]. Dichloromethane (CH_2Cl_2) and tetrahydrofuran (THF) for anhydrous reactions were dried over CaH_2 and distilled prior to use. Solvents used for extraction and chromatography such as CH_2Cl_2, hexane, ethanol, and ethyl acetate were commercial grade and distilled before use. Solvents used for extraction and chromatography such as CH_2Cl_2, hexane, ethanol and ethyl acetate were commercial grade and distilled before use. Deionized water was distilled and used in extraction procedures. MilliQ water was used to prepare stock metal ions and fluorophore solutions for UV Visible and fluorescence experiments. Reactions were mostly carried out under positive pressure of N_2 gas filled in rubber balloons. Thin layer chromatography (TLC) was carried out using Merck 60 F254 plates with a thickness of 0.25 mm. Column chromatography was performed on Merck silica gel 60 (70-230 mesh).

2.2 Analytical instruments

The HRMS was undertaken on an electrospray ionization mass spectrometer (microTOF, Bruker Daltonics). Fourier transform infrared spectra (FTIR) were obtained on Nicolet 6700 FTIR spectrometer equipped with a mercury-cadmium telluride (MCT) detector (Nicolet, USA). ^{1}H NMR spectra were recorded on Varian Mercury 400 MHz NMR spectrometer (Varian, USA). ^{13}C-NMR spectra were recorded at 100 MHz on Bruker NMR spectrometer. The UV-Visible spectra were acquired from a Varian Cary 50 UV-Vis spectrophotometer (Varian, USA) using milliQ water or acetonitrile as a solvent. Fluorescence emission spectra were obtained by using Perkin Elmer precisely LS 45 Luminescence Spectrometer (PerkinElmer, UK) for metal ion sensing and using a Varian Cary Eclipse spectrofluorometer (Varian, USA) for photophysical property studies.

2.3 Synthetic procedures

2.3.1 Synthesis of 1,4-dihydropyridine (R-DHP-OH)



Me-DHP-OH; R=Me, 70%
Et-DHP-OH; R=Et, 83%

Into the solution of ethanolamine (1 equiv) in CH_2Cl_2, alkyl propiolate (1.2 equiv) was slowly added and the reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. The mixture was evaporated *in vacuo* to obtain the corresponding *N*-aliphatic β-amino acrylates.

This crude *N*-substituted β-amino acrylates (1 equiv) was dissolved in dried CH_2Cl_2 (1.66 M) in an ice bath without any purification. Then $TiCl_4$ (0.2 equiv) was rapidly added and stirred for overnight at room temperature under nitrogen atmosphere. Then the solution was quenched with cold deionized water (25 mL). After that, the mixture was extracted with CH_2Cl_2 (25 mL). The organic portions were combined and neutralized by addition of 0.1 M aq $NaHCO_3$ solution. The organic phase was washed three times with cold deionized water (3x25 mL), dried over $MgSO_4$ anhydrous, and evaporated under reduced pressure. The crude obtained product was purified by column chromatography (EtOAc/hexane = 1:50 to 1:3) to provide the corresponding 1,4-dihydropyridine (R-DHP-OH).

Diethyl-4-(2-ethoxy-2-oxoethyl)-1-(2-hydroxyethyl)-1,4-dihydropyridine-3,5-dicarboxylate, (Et-DHP-OH)

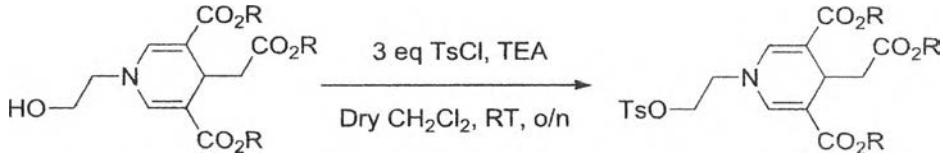
Synthesized according to the above general procedure from ethanolamine (1.01 g, 16.6 mmol) and ethyl propiolate (1.95 g) as pale yellow oil (1.62 g, 83%); R_f (EtOAc) 0.46; ν_{max} (neat) 3465, 2984, 1689, 1657, 1575, 1409, 1222, 1177, 1056 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.17 (1H, s, *CH*=C), 4.26-4.12 (4H, m, $CO_2CH_2CH_3$, and CH_2CH_2OH), 4.07 (1H, t, *J* 4 Hz, $CHCH_2CO_2$), 4.02 (2H, q, *J* 7 Hz, $CH_2CO_2CH_2CH_3$), 3.85 (1H, t, *J* 7 Hz,), 3.75 (2H, dd, *J* 10, 6 Hz), 3.43-3.37 (2H, m, CH_2CH_2OH), 2.64 (2H, d, *J* 4 Hz, $CH_2CO_2CH_2CH_3$), 1.28 (6H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 1.18 (3H, t, *J* 7 Hz, $CH_2CO_2CH_2CH_3$); δ_C

(100 MHz, $CDCl_3$): 173.8, 166.8, 140.3, 105.3, 61.7, 60.3, 60.1, 57.6, 39.1, 28.6, 14.4, 14.0 HRMS (ESI): $[M+Na]^+$, found 378.1522. $C_{17}H_{25}NNaO_7^+$ requires 578.1529.

Dimethyl-1-(2-hydroxyethyl)-4-(2-methoxy-2-oxoethyl)-1,4-dihydropyridine-3,5-dicarboxylate, (Me-DHP-OH)

Synthesized according to the above general procedure from ethanolamine (1.01 g, 16.6 mmol) and methyl propiolate (1.67 g) as pale yellow oil (1.37 g, 70%); R_f (EtOAc) 0.43; ν_{max} (neat) 3514, 3058, 2981, 2951, 2849, 1701, 1581, 1437, 1262, 1185, 741 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.18 (1H, s, *CH*=C), 4.07 (1H, t, *J* 4 Hz, *CH*CH_2CO_2), 3.75 (2H, t, *J* 4 Hz, *CH*$_2$OH), 3.72 (3H, s, CCO_2CH_3), 3.58 (3H, s, $CH_2CO_2CH_3$), 3.42 (2H, t, *CH*$_2$N), 2.66 (2H, d, *J* 4 Hz, *CH*$_2CO_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$): 174.1, 167.0, 140.5, 105.3, 61.7, 57.6, 51.4, 38.8, 28.7 HRMS (ESI): $[M+Na]^+$, found 336.1034. $C_{14}H_{19}NNaO_7^+$ requires 336.1054.

2.3.2 Synthesis and of 1,4-dihydropyridine tosylate (R-DHP-OTs)



Me-DHP-OTs ; R=Me , 20%
Et-DHP-OTs ; R=Et , 50%

R-DHP-OH in CH_2Cl_2 (~0.03 M) was added TEA (1.5 equiv). Then, TsCl (3 equiv) was slowly added to mixture at 0 °C and the reaction mixture was stirred for overnight at room temperature under nitrogen atmosphere. After the solvent evaporation, the cold deionized water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3x10 mL). The organic phase was dried over anhydrous $MgSO_4$. The obtained crude product was purified by column chromatography (EtOAc/hexane = 40:60) to provide the corresponding tosyl 1,4-dihydropyridine (R-DHP-OTs).

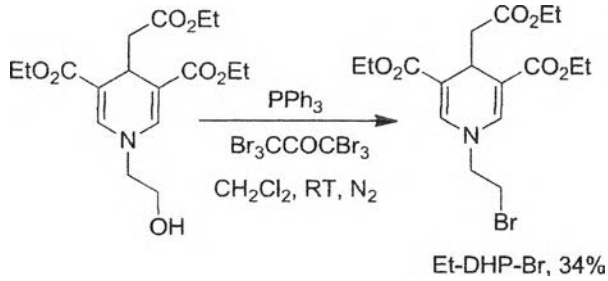
Diethyl-4-(2-ethoxy-2-oxoethyl)-1-(2-(tosyloxy)ethyl)-1,4-dihydropyridine-3,5-dicarboxylate (Et-DHP-OTs),

Synthesized from Et-DHP-OH (100 mg, 0.28 mmol) and TsCl (161 mg, 3 equiv) according to the above general procedure, to afford yellow liquid oil (144 mg, 50%); R_f (40% EtOAc/hexane) 0.35; ν_{max} 2978, 1695, 1580, 1360, 1234, 1174, 1068 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.69 (1H, d, *J* 4 Hz, *CH*CS), 7.25 (1H, d, *J* 4 Hz, *CH*CCH_3), 6.90 (1H, s, *CH*=C), 4.13-4.10 (4H, m, , $CO_2CH_2CH_3$, and CH_2CH_2OH), 3.97 (1H, t, *J* 4 Hz, *CH*CH_2CO_2), 3.94 (2H, q, *J* 7 Hz, $CH_2CO_2CH_2CH_3$), 3.49 (2H, t, *J* 4 Hz, CH_2N), 2.37 (3H, s, CH_3 of tosyl), 2.34 (2H, d, *J* 4 Hz, $CH_2CO_2CH_2CH_3$), 1.23 (6H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 1.12 (3H, t, *J* 7 Hz, $CH_2CO_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$): 169.1, 163.9, 142.8, 136.1, 130.1, 127.3, 125.3, 104.5, 65.4, 57.8, 57.6, 50.5, 38.2, 26.9, 19.3, 12.0, 11.8. HRMS (ESI): $[M+Na]^+$, found 532.1582. $C_{24}H_{31}NNaO_9S^+$ requires 532.1617.

Dimethyl-4-(2-methoxy-2-oxoethyl)-1-(2-(tosyloxy)ethyl)-1,4-dihydropyridine-3,5-dicarboxylate, (Me-DHP-OTs)

Synthesized between Me-DHP-OH (100 mg, 0.32 mmol) and TsCl (183 mg, 3 equiv) according to the above general procedure, using CH_2Cl_2 as solvent, to afford yellow liquid oil (30 mg, 20%); R_f (40% EtOAc/hexane) 0.32; ν_{max} 2978, 1695, 1580, 1360, 1234, 1174, 1068 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.75 (1H, d, *J* 8 Hz, *CH*CS), 7.31 (1H, d, *J* 8 Hz, *CH*CCH_3), 7 (1H, s, *CH*=C), 4.22 (2H, t, *J* 4 Hz, CH_2SO_3), 4.17 (2H, t, *J* 6 Hz, CH_2N), 4.04 (1H, t, *J* 6 Hz, *CH*CH_2CO_2), 3.73 (3H, s, CH_3CO_2), 3.56 (3H, s, $CH_3CO_2CH_2$), 2.43 (3H, s, CH_3 of tosyl), 2.41 (2H, d, *J* 4 Hz, $CH_2CO_2CH_2CH_3$); δ_C (100 MHz, $CDCl_3$): 171.8, 166.8, 145.4, 138.8, 130.1, 128.1, 106.6, 67.6, 53.0, 51.4, 51.2, 40.4, 29.1, 21.6 HRMS (ESI): $[M+Na]^+$, found 490.1142. $C_{24}H_{31}NNaO_9S^+$ requires 490.1140.

2.3.3 Synthesis of brominated 1,4-dihydropyridine (Et-DHP-Br)



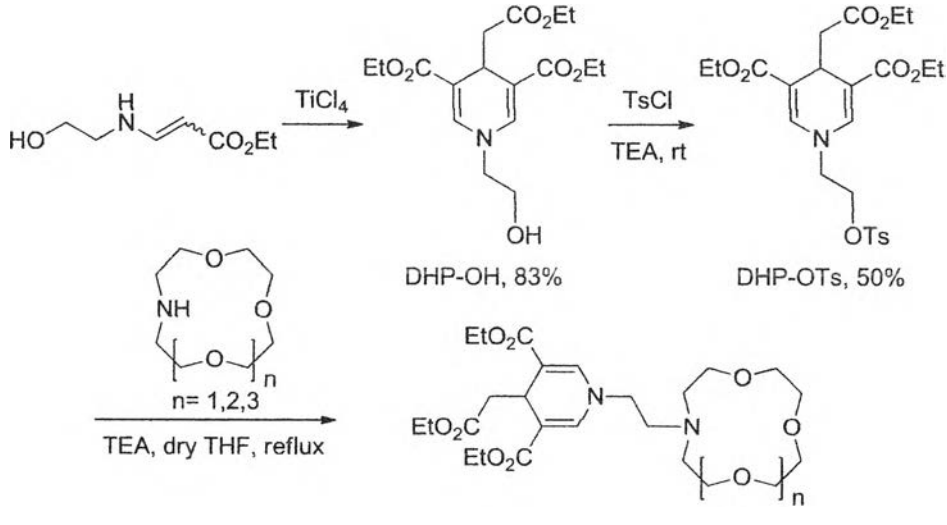
Et-DHP-Br, 34%

Et-DHP-OH and PPh_3 (1.5 equiv) in CH_2Cl_2 (0.56 M) was added hexabromoacetone ($Br_3CCOCBr_3$) as brominating agent. Then, the mixture was stirred at room temperature under nitrogen atmosphere in 2 hours. After the solvent evaporation, the cold deionized water (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3x10 mL). The organic phase was dried over anhydrous $MgSO_4$. The obtained crude product was purified by column chromatography (EtOAc/hexane = 40:60) to provide the corresponding bromo 1,4-dihydropyridine (Et-DHP-Br).

Diethyl-1-(2-bromoethyl)-4-(2-ethoxy-2-oxoethyl)-1,4-dihydropyridine-3,5-dicarboxylate, (Et-DHP-Br)

Synthesized from Et-DHP-OH (100 mg, 0.28 mmol), PPh_3 (111 mg, 1.5 equiv), and $Br_3CCOCBr_3$ (224 mg, 1.5 equiv) according to the above general procedure, to afford yellow liquid oil (39.8 mg, 34%); R_f (EtOAc) 0.62; ν_{max} 3052, 2982, 2308, 1702, 1588, 1265, 742 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.07 (1H, s, C*H*=C), 4.09-4.17 (5H, m, $CHCH_2CO_2$ and CH_2OCO_2C), 3.97 (2H, q, *J* 8 Hz, $CH_2OCO_2CH_2$), 3.63 (2H, t, *J* 6 Hz, CH_2N), 3.41 (2H, t, *J* 6 Hz, CH_2Br), 2.42 (2H, d, *J* 6 Hz, CH_2CO_2), 1.22 (3H, t, *J* 8 Hz, $CH_3CH_2CO_2C$), 1.14 (3H, t, *J* 8 Hz, $CH_3CH_2CO_2CH_2$); δ_C (100 MHz, $CDCl_3$): 171.1, 166.6, 138.6, 107.0, 60.2, 60.0, 55.8, 40.5, 29.6, 29.3, 14.3, 14.2.

2.3.4 Synthesis of 1,4-dihydropyridine azacrown ether derivatives (Et-DHP-AC(1-3))



Et-DHP-AC(1) ; n=1, 44%
Et-DHP-AC(2) ; n=2, 39%
Et-DHP-AC(3) ; n=3, 33%

Et-DHP-AC(1-3) were prepared by the nucleophilic substitution (S_N2) reaction with various sizes of azacrown ether (n=1-3). Et-DHP-OTs (300 mg, 0.59 mmol) in THF (10 mL) was added with TEA (2.5 equiv), followed by the addition of azacrown ether (n=1-3) (1.5 equiv). The reaction mixture was then stirred for 2 days at 66°C (refluxing temperature) under nitrogen atmosphere. After that the solution was evaporated to eliminate THF by rotary evaporator, the crude product was purified by column chromatography ($EtOH/CH_2Cl_2$ = 2:98) to provide the corresponding 1,4-dihydropyridine azacrown ether (Et-DHP-AC(1-3)).

Diethyl-1-(2-(1,4,7-trioxa-10-azacyclododecan-10-yl)ethyl)-4-(2-ethoxy-2-oxoethyl)-1,4-dihydropyridine-3,5-dicarboxylate (Et-DHP-AC(1)),

Synthesized from Et-DHP-OTs and azacrown ether (n=1) (124 mg) according to the above general procedure, to afford yellow liquid (133 mg, 44%); R_f (10% $EtOH/CH_2Cl_2$) 0.37; ν_{max} 3052, 2985, 1723, 1698, 1575, 1264, 1080 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.14 (2H, s, *CH*=C), 4.19-4.14 (3H, m, CH_2OCO_2 and $CHCH_2CO_2$), 4.01 (2H, q, *J* 7 Hz, $CH_2OCO_2CH_2$), 3.67-3.57 (4H, m, CH_2OCH_2), 3.37 (2H, t, *J* 6 Hz, CH_2N CH=C), 2.76-2.66 (4H, m, CH_2N CH_2), 2.42 (2H, d, *J* 4 Hz, CH_2CO_2), 1.27 (3H, t, *J* 7 Hz, $CH_3CH_2CO_2C$); 1.18 (3H, t, *J* 7 Hz, $CH_3CH_2CO_2CH_2$), δ_C (100 MHz, $CDCl_3$): 171.5, 167.0, 142.8, 139.6, 106.0, 71.4, 70.3, 60.0, 59.9, 56.7, 55.6, 53.3, 41.2, 29.4, 14.4, 14.1. HRMS (ESI): $[M+H]^+$, found 513.2843. $C_{25}H_{41}N_2O_9^+$ requires 513.2812.

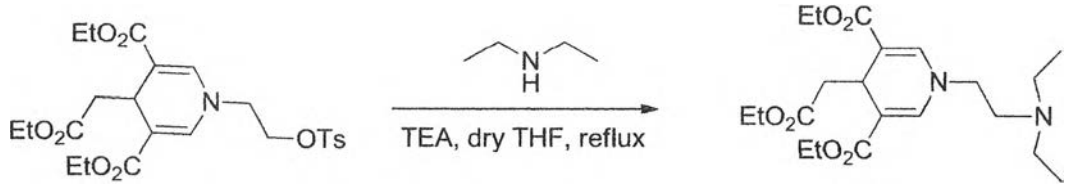
Diethyl-1-(2-(1,4,7,10-tetraoxa-13-azacyclopentadecan-13-yl)ethyl)-4-(2-ethoxy-2-oxoethyl)-1,4-dihydropyridine-3,5-dicarboxylate (Et-DHP-AC(2)),

Synthesized from Et-DHP-OTs and azacrown ether (n=2) (155 mg) according to the above general procedure, to afford yellow liquid oil (128 mg, 39%); R_f (10% $EtOH/CH_2Cl_2$) 0.39; ν_{max} 3052, 2985, 1723, 1701, 1584, 1264, 1077 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.11 (2H, s, *CH*=C), 4.18-4.13 (4H, m, CH_2OCO_2 and $CHCH_2CO_2$), 3.99 (2H, q, *J* 7 Hz, $CH_2OCO_2CH_2$), 3.67-3.57 (4H, m, CH_2OCH_2), 3.33 (2H, t, *J* 6 Hz, CH_2N CH=C), 2.78-2.70 (4H, m, CH_2N CH_2), 2.41 (2H, d, *J* 4 Hz, CH_2CO_2), 1.26 (6H, t, *J* 7 Hz, $CH_3CH_2CO_2C$); 1.17 (3H, t, *J* 7 Hz, $CH_3CH_2CO_2CH_2$), δ_C (100 MHz, $CDCl_3$): 171.7, 166.9, 142.8, 139.6, 106.0, 70.9, 70.4, 70.2, 60.0, 59.9, 56.3, 56.7, 56. 55.1, 53.1, 41.2, 29.4, 14.4, 14.2. HRMS (ESI): $[M+H]^+$, found 557.3068. $C_{27}H_{45}N_2O_{10}^+$ requires 557.3074.

Diethyl-1-(2-(1,4,7,10,13-pentaoxa-16-azacyclooctadecan-16-yl)ethyl)-4-(2-ethoxy-2-oxoethyl)-1,4-dihydropyridine-3,5-dicarboxylate (Et-DHP-AC(3)),

Synthesized between Et-DHP-OTs and azacrown ether (n=3) (186 mg) according to the above general procedure, using THF as solvent, to afford yellow liquid oil (117 mg, 33%); R_f (10% EtOH/CH_2Cl_2) 0.43; ν_{max} 3052, 2985, 1723, 1698, 1578, 1264, 1116 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.17 (2H, s, *CH*=C), 4.21-4.15 (4H, m, CH_2OCO_2 and $CHCH_2CO_2$), 4.01 (2H, q, *J* 7 Hz, $CH_2OCO_2CH_2$), 3.67-3.57 (4H, m, CH_2OCH_2), 3.42 (2H, t, *J* 6 Hz, CH_2NCH=C), 2.86-2.76 (4H, m, CH_2NCH_2), 2.42 (2H, d, *J* 5 Hz, CH_2CO_2), 1.28 (6H, t, *J* 7 Hz, $CH_3CH_2CO_2C$); 1.20 (3H, t, *J* 7 Hz, $CH_3CH_2CO_2CH_2$), δ_C (100 MHz, $CDCl_3$): 171.8, 166.8, 142.8, 139.5, 105.7, 70.7, 70.6, 70.5, 70.3, 60.0, 59.9, 55.6, 54.6, 53.2, 41.1, 29.3, 14.4, 14.2. HRMS (ESI): $[M+H]^+$, found 601.3298. $C_{29}H_{49}N_2O_{11}^+$ requires 601.3336.

2.3.5 Synthesis of diethyl-1-(2-(diethylamino)ethyl)-4-(2-ethoxy-2-oxoethyl)-1,4-dihydropyridine-3,5-dicarboxylate, (Et-DHP-NEt_2)



Et-DHP-NEt_2, 29%

Et-DHP-NEt_2 was prepared by the nucleophilic substitution (S_N2) reaction with diethylamine (DEA) (73 µL). Et-DHP-OTs (300 mg, 0.59 mmol) in THF (10 mL) was added TEA (2.5 equiv) and then DEA (1.5 equiv) was added in the mixture of compound and was stirred for 2 days at 66 ^{o}C (refluxing temperature) under nitrogen atmosphere. After that the solution was evaporated to eliminate THF by rotary evaporator. The crude obtained product was purified by column chromatography (EtOH/CH_2Cl_2 = 2:98) to provide the corresponding 1,4-dihydropyridine liked with DEA (Et-DHP-NEt_2) as afford yellow liquid (70 mg, 29%); R_f (10% EtOH/CH_2Cl_2) 0.30; ν_{max} 2975, 2932, 1732, 1701, 1578, 1206, 1077 cm^{-1}; δ_H (400 MHz, $CDCl_3$): 7.17 (2H, s, *CH*=C), 4.22-4.16 (4H, m, CH_2OCO_2 and $CHCH_2CO_2$), 4.03 (2H, q, *J* 6 Hz, $CH_2OCO_2CH_2$), 3.35 (2H, t, *J* 7 Hz, CH_2NCH=C), 2.60 (2H, t, *J* 6 Hz, CH_2CH_2N), 2.52 (2H, q, *J* 8 Hz, NCH_2CH_3), 2.43 (2H, d, *J* 6 Hz, $CHCH_2CO_2$), 1.28 (3H, t, *J* 7 Hz, $CO_2CH_2CH_3$); 1.20 (3H, t, *J* 7 Hz, $CH_2CO_2CH_2CH_3$), 1.00 (3H, t, *J* 7 Hz, NCH_2CH_3), δ_C (100 MHz, $CDCl_3$): 171.7, 167.0,

139.8, 105.8, 60.0, 59.9, 53.4, 53.3, 47.3, 41.2, 29.4, 14.4, 14.2, 12.0. HRMS (ESI): $[M+H]^+$, found 411.2507. $C_{21}H_{35}N_2O_6^+$ requires 411.2495.

2.4 Analytical experiment

The photophysical property study, the sensing studied with metal ion and nitroaromatic compounds sensing were all achieved in milliQ water.

2.4.1 Photophysical property study

The stock solutions of fluorophore were prepared by dissolving 10 μmol of Et-DHP-AC(1-3) and Et-DHP-NEt_2 in milliQ water (50 mL) to get the concentration of 200 μM of Et-DHP-AC(1-3) and Et-DHP-NEt_2 stock solutions.

i) UV-Visible spectroscopy

The UV-Visible absorption spectra of the stock solution of all fluorophores were recorded from 200 nm to 500 nm at ambient temperature.

ii) Fluorescence spectroscopy

The stock solutions of all fluorophores were diluted into the concentration of 10 μM. The emission spectra of fluorophores were recorded from 380 nm to 600 nm at ambient temperature using an excitation wavelength at 367 nm (Et-DHP-AC(1)), 369 nm (Et-DHP-AC(2)), 362 nm (Et-DHP-AC(3)), and 360 nm (Et-DHP-NEt_2) gained by UV absorption wavelength.

iii) Molar extinction coefficient (ε)

The molar extinction coefficient (ε) of Et-DHP-AC(1-3) and Et-DHP-NEt_2 were calculated from the UV-Visible absorption spectra of fluorophore at various concentrations in milliQ water. The maximum absorbance of all fluorophores should not be more than value of 1. The wavelength of maximum absorbance (λ_{max}) of each compound was plotted against the concentrations at the respective excitation wavelengths (λ_{ex}). Each plot should be a linear line that runs through the origin position (0,0). The molar extinction coefficient (ε) can also be calculated from plotting of absorption maximum (A) vs concentration (C) represented into the following Beer-Lambert law equation:

$$A = \varepsilon bC$$

iv) Fluorescence quantum yield (Φ_F)

The fluorescence quantum yield of Et-DHP-AC(1-3) and Et-DHP-NEt_2 were performed in milliQ water by using Quinine sulfate in 0.1 M H_2SO_4 (Φ=0.54) as a reference. The UV-Visible absorption spectra of fluorophore and reference at various concentrations were recorded. The maximum absorbance of all fluorophores should not be more than value of 0.1 in order to prevent the interaction among themselves at high concentration. The fluorescence emission spectra of the solutions using appropriate excitation wavelengths selected were recorded based on the wavelength of maximum absorbance (λ_{max}) of each compound. The graphs of integrated fluorescence intensity were plotted against the absorbance at the respective excitation wavelengths. Each plot should be a linear line with 0 interception and gradient.

Moreover, the fluorescence quantum yield (Φ_F) 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} signify the fluorescence quantum yield of a standard reference which used quinine sulfate in 0.1 M H_2SO_4 (Φ=0.54) and Φ_X is the fluorescence quantum yield of sample and η is the refractive index of the solvent.

2.4.2 Metal ion sensor

Each of stock solution metal ion was prepared in milliQ water by using metal acetate except ferric nitrate (Fe^{3+}), ferrous sulfate (Fe^{2+}) and cadmium sulfate (Cd^{2+}) into a concentration of 2 mM.

To attain the fluorescence quenching profile, the Et-DHP-AC(3) and metal solution were diluted and mixed with metal ion in a ratio of 1:10 and allowed to stand at room temperature for 20 min and recorded spectra from 380 nm to 600 nm at ambient temperature by fluorescence spectrophotometer. Using the concentration of fluorophore (10 μM) and metal ions (100 μM) can accomplish the visible fluorescence response and photographed under black light. To gain the

fluorescence titration spectra, the mixtures of fluorophore and metal was prepared by 0 to 60 equiv and allowed to stand at room temperature for 20 min. UV–vis metal binding titration was prepared as same as fluorescence titration but increasing the concentration of fluorophore to 30 μM, recorded spectra by UV-vis spectrophotometer. The mixture of fluorophore/Au^{3+}/others metal ions with ratio 1/10/50 was used to investigate competitive experiments in the Et-DHP-AC(3)·Au^{3+} system.

^1H NMR experiment: compound Et-DHP-AC(3) (1.5 mg, 2.5 μmol) was dissolved in a D_2O (0.4 mL). Into this aqueous solution, $HAuCl_4$ (0.85 mg, 1 equiv) was added and the solution was shaken thoroughly. ^1H NMR experiment was then conducted immediately with NMR spectrometer (Bruker, 400 MHz). The spectra of this experiment at the times of 0 to 25 minutes were illustrated to the change in chemical shifts of the Et-DHP-AC(3).

2.4.3 Nitroaromatic sensor

Stock solution nitroaromatic compounds were prepared at concentration of 100 μM in milliQ water. Concentrations of Et-DHP-AC(3) and Et-DHP-NEt_2 was adjusted to 10 μM in milliQ water and used as stock solutions. The final volume of the mixtures was adjusted to 1 mL to afford the final concentration of 10 μM for the fluorophore and 100 μM for the nitroaromatic. After the solution was mixed, fluorescence spectra were measured with an excitation wavelength of 362 nm at room temperature. Fluorescence spectra were recorded from 380 to 600 nm.