Synthesis and Characterization of Dimaleimide Fluorogens Designed for Specific Labeling of Proteins

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Supporting Information

Experimental Section

Synthesis

Materials

The following material were obtained from Aldrich and were used without further purification: 2-hydroxy-5-nitrobenzaldehyde, *N*-acetylglycine, maleic anhydride, *p*-nitrophenylacetic acid, L-aspartic acid dimethyl ester hydrochloride, citraconic anhydride, dimethyl L-tartrate, dimethyl malonate. All reactions were carried out under an atmosphere of dry nitrogen employing conventional bench-top techniques except for reactions with thiols. ^{13}C-NMR and ^{1}H-NMR spectra were recorded on AMXR400 or AMX300 spectrometers and were referenced to the residual proton or ^{13}C signal of the solvent. Mass spectra were determined by FAB+ ionization on an AutoSpecQ spectrometer at the Regional Mass Spectrometry Centre at the Université de Montréal. Infrared spectra were recorded on a Perkin-Elmer (FTIR) spectrometer. Melting points (uncorrected) were determined on a Unimelt Tomas-Hoover or on a Gallenkamp melting point apparatus.

Methods

3-Acetamido-6-nitrochromen-2-one (1)

A dry 100-mL round bottomed flask was charged with 7.11 g (42.5 mmol) of 2-hydroxy-5-nitrobenzaldehyde. To this flask were then added *N*-acetylglycine (4.98 g, 42.5 mmol) and acetic anhydride (40.1 mL, 0.43 mol). In small aliquots, 1.71 g (42.5 mmol)of sodium hydride (60% dispersion in mineral oil) was added to the flask. Reactants were observed to dissolve and after 2-5 minutes, precipitation occurred. The reaction mixture was stirred for 20 h and 7.11 mL of water were added. Following the addition of 43 mL acetic acid, the mixture was cooled to

4°C for 4 h. The resulting precipitate was filtered and washed liberally with cold glacial acetic acid. The acetic acid was then removed as an azeotrope upon addition of 250 mL toluene and rotary evaporation to dryness, three times. The final residue was dried under vacuum to give product **1** as a beige powder (6.40 g, 25.8 mmol) in a yield of 61%. mp 277-279 °C (lit[1] 278 °C) FTIR (KBr) (cm^{-1}) 3350, 3050, 1710, 1680, 1600, 1500, 1420, and 1335. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 9.93 (s, 1H), 8.76 (s, 1H), 8.73 (d, *J* = 2.5 Hz, 1H), 8.28 (dd, *J* = 2.8 Hz, *J* = 9.1 Hz, 1H), 7.60 (d, *J* = 9.1 Hz, 1H), 2.19 (s, 3H). ^{13}C-NMR (DMSO-d_6): δ (ppm) 171.47, 157.69, 145.07, 127.11, 124.96, 124.52, 122.79, 121.52, 118.26, 25.00. HRMS expected: 249.0524; found: 249.0534. Elemental analysis for $C_{11}H_8N_2O_5$, expected: C, 53.23; H, 3.25; :N, 11.27; found: C, 53.19; H, 3.34; N, 11.06.

3-Acetamido-6-aminochromen-2-one (2)

Palladium 10 wt % on activated carbon (0.322 g, 1/10th the mass of **1**) was placed in a 1-L round-bottom flask and the flask was purged with nitrogen. A solution of sodium borohydride (1.21g, 32.9 mmol) in 22.6 mL water was added dropwise. A suspension of compound **1** (3.20 g, 12.9 mmol) in methanol (775 mL) was then added over a two hour period and allowed to stir at room temperature an additional 30 min, when the reaction mixture was filtered through Celite and the solvent was removed by rotary evaporation. Water (750 mL) was added to the residue and the solution was cooled to 4°C for 20 h. The resulting precipitate was filtered and rinsed with cold distilled water. The solid was recrystallized from ethanol to give **2** as a yellow solid (1.59 g, 7.3 mmol) in 57 % yield. mp 250 – 252 °C (lit[2] 252 – 253 °C). FTIR (KBr) (cm^{-1}) : 3410, 3310, 1700, 1650, 1630. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 9.65 (s, 1H), 8.40 (s, 1H), 7.10 (dd, *J* = 9.1 Hz, *J* = 2.7 Hz, 1H), 6.73 (d, *J* = 9.1 Hz, 1H), 6.68 (d, *J* = 2.7 Hz, 1H), 5.19 (bs, 2H), 2.14 (s,

3H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 171.12, 158.78, 146.92, 142.52, 125.26, 124.98, 120.82, 117.54, 117.15, 110.66, 24.93. HRMS expected, 219.0777; found, 219.0770. Elemental analysis for $C_{11}H_{10}N_2O_3$: expected : C, 60.55; H, 4.62; N, 12.84; found : C, 60.15; H, 4.50; N, 12.62.

3,6-Diaminochromen-2-one (3)

To a solution composed of 12.63 mL of 12 M hydrochloric acid in 6.32 mL ethanol was added 0.919 g (4.2 mmol) of compound **2**. The solution was heated to reflux for 30 min and then cooled to 4 °C overnight. The resulting precipitate was removed by filtration and dissolved in a minimal volume of distilled water. The solution was neutralized by addition of 25% ammonium hydroxide and cooled at 4 °C for three hours. The resulting precipitate was removed by filtration and recrystallized from water to give a yellow compound (**3**) (0.484 g, 2.7 mmol) in 65% yield. mp 183–186 °C (lit[1] 183 - 184°C). FTIR (KBr) (cm^{-1}) : 3420, 3340, 1690, 1635. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 6.95 (d, *J* = 8.5 Hz, 1H), 6.56 (s, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 6.46 (dd, *J* = 10.0 Hz, *J* = 2.6 Hz, 1H), 5.48 (bs, 2H), 5.00 (bs, 2H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 159.95, 146.50, 141.19, 134.13, 123.04, 116.75, 113.70, 109.30, 108.42. HRMS expected : 177.0592; found : 177.0598.

3,6-Dimaleimidylchromen-2-one (4)

Diamine **3** (200 mg, 1.14 mmol) and maleic anhydride (335 mg, 3.42 mmol) were placed in a dry 50-mL round bottom flask. Chloroform (11.5 mL) was added and the solution was heated to reflux for 20 h. The mixture was then filtered and the recovered solid was rinsed liberally with chloroform and then dried under vacuum. To this solid was added acetic anhydride (9 mL, 96

mmol) and sodium acetate (35 mg, 0.427 mmol) and the reaction was stirred vigorously for another 30 min. The mixture was then cooled to 4 °C for 4 h and filtered. The beige solid thus obtained was dried over vacuum to give compound **4** in 20% yield (76 mg, 0.226 mmol). mp : >296 °C (dec). FTIR (KBr) (cm^{-1}) : 1700, 1620. ^{1}H-NMR ($CDCl_3$) : δ (ppm) 7.78 (s,1H), 7.66 (dd, *J* = 9.8 Hz, *J* = 2.5 Hz, 1H), 7.63 (d, *J* = 2.5 Hz, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 6.93 (s, 2H), 6.91 (s, 2H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 1699.99, 169.06, 156.80, 151.50, 143.62, 135.82, 134.89, 131.61, 128.63, 127.11, 118.60, 118.13, 117.58. HRMS : expected, 337.0463; found, 337.0461.

3,6-Di-(3'-methoxycarbonylmaleimidyl)chromen-2-one (5)

To a suspension of diamine **3** (268 mg, 1.52 mmol) in dry chloroform (15.4 mL) was added á-carbomethoxymaleic anhydride (712 mg, 4.56 mmol). The solution was heated to reflux for 20 h, then cooled to room temperature and filtered. The solid thus obtained was rinsed liberally with chloroform and dried under reduced pressure. It was then added to 12 mL acetic anhydride and 47 mg of sodium acetate (0.56 mmol). The mixture was then heated to 100 °C for 90 min and then allowed to cool to room temperature for 24 h. The resulting precipitate was recovered by filtration and rinsed with water, giving the desired product **5** in 22 % yield (151 mg, 0.33 mmol). mp >230 °C (dec). FTIR (KBr) (cm^{-1}) : 1720, 1670. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 8.24 (s, 1H,), 8.04 (s, 2H), 7.66 (dd, *J* = 8.97 Hz, *J* = 2.43 Hz, 1H), 7.42 (d, *J* = 8.96 Hz, 1H), 7.40 (d, *J* = 2.42 Hz, 1H), 4.04 (s, 3H), 4.00 (s, 3H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 171.02, 163.38, 132.09, 159.92, 145.83, 142.80, 142.31, 135.32, 128.06, 124.83, 120.92, 119.92, 119.27, 117.16, 48.83. HRMS expected for $C_{21}H_{12}N_2O_{10}$: 452.0492; found: 452.0491.

3,6-Di-(3'-methylmaleimidyl)chromen-2-one (6)

To a suspension of diamine **3** (268 mg, 1.52 mmol) in 15.4 mL of dry chloroform was added citraconic anhydride (410 µL, 4.56 mmol). The mixture was heated to reflux for 20 h. The mixture was cooled to room temperature and filtered, and the recovered solid was rinsed well with chloroform. After drying, it was added to a solution of sodium acetate (47 mg, 0.56 mmol) in acetic anhydride (12 mL). The mixture was heated to 100 °C for 90 min and then cooled to room temperature and allowed to stand for 24 h. The resulting precipitate was then filtered and rinsed with water to give the desired product **6** in 20% yield (147 mg, 0.3 mmol). mp >230 °C (dec). FTIR (KBr) (cm^{-1}) : 1720, 1654. ^{1}H-NMR ($CDCl_3$) : δ (ppm) 8.52 (s, 1H), 7.88 (d, *J* = 2.29 Hz, 1H), 7.63 (s, 1H), 7.52 (dd, *J* = 9.26 Hz, *J* = 2.29 Hz, 1H), 7.45 (s, 1H), 7.34 (d, *J* = 8.83 Hz, 1H), 2.07 (d, *J* = 6.92 Hz, 3H), 1.91 (d, *J* = 7.02 Hz, 3H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 170.00, 169.02, 158.90, 146.22, 137.32, 126.04, 122.06, 120.43, 118.24, 117.16, 24.80. HRMS expected for $C_{19}H_{12}N_2O_6$: 364.0695; found: 364.0693.

3,6-Di(3'-ethylthiosuccinimidyl)chromen-2-one (7)

To a suspension of **4** (40 mg, 0.12 mmol) in 3 mL dry chloroform was added 27 µL (0.36 mmol) of ethanethiol. The solution was stirred at room temperature for 12 h, then concentrated under reduced pressure and purified by flash chromatography (30:70 EtOAc:hexane). Compound **7** was thus obtained as a yellow solid in 85% yield (47 mg, 0.10 mmol). mp 128-131 °C. FTIR (KBr) (cm^{-1}): 3000, 1700, 1620, 600. ^{1}H-NMR ($CDCl_3$) : δ (ppm) 7.46 (m, 2H), 7.42 (d, *J* = 11.0 Hz, 1H) 7.39 (s, 1H), 3.90 (d, *J* = 5.6 Hz, 1H), 3.88 (d, *J* = 5.6 Hz, 1H), 3.35 (q, *J* = 9.7 Hz, 2H), 2.94 (m, 2H), 2.80 (m, 2H), 2.69 (d, *J* = 5.6 Hz, 1H), 2.64 (d, *J* = 5.6 Hz, 1H), 1.29 (t, *J* = 8.0 Hz, 6H). ^{13}C-NMR ($CDCl_3$) : δ (ppm) 175.20, 173.42, 167.90, 155.98, 151.07, 136.55,

130.14, 128.85, 128.25, 125.39, 118.72, 117.35, 38.72, 35.96, 26.04, 20.40, 14.03. HRMS calculated: 461.0851; found: 461.0849. Elemental analysis for $C_{21}H_{20}N_2O_6S_2$ expected: C, 54.77; H, 4.38; N, 6.08; S, 13.92; found: C, 54.49; H, 4.35; N, 5.99; S, 13.50.

3,6-Di-(3'-ethylthio-4'-methoxycarbonylsuccinimidyl)chromen-2-one (8)

The reaction of 54 mg (0.12 mmol) of compound **5** in 3 mL dry DMSO with 27 µL ethanethiol (0.36 mmol) was carried out according to the same protocol used to obtain compound **7**, giving compound **8** in 83 % yield (58 mg, 0.1 mmol). FTIR (KBr) (cm^{-1}) : 3000, 1727, 1622, 600. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 8.24 (s, 1H), 7.53 (d, *J* = 2.63, 1H), 7.36 (dd, *J* = 9.24 Hz, *J* = 2.67 Hz, 1H), 7.24 (d, *J* = 9.24 Hz, 1H), 4.38 (d, 1H), 4.32 (s, 1H), 4.00 (s, 3H), 3.98 (d, *J* = 5.6 Hz, 3H), 3.92 (d, *J* = 5.4 Hz, 1H), 3.87 (d, *J* = 5.6 Hz, 1H), 2.80 (m, 4H), 1.30 (t, *J* = 7.24 Hz, 6H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 175.06, 172.67, 168.06, 158.44, 150.63, 137.28, 128.83, 128.32, 124.22, 119.69, 118.47, 117.21, 50.01, 46.23, 42.16, 39.12, 14.24, 12.02. HRMS expected for $C_{25}H_{24}N_2O_{10}S_2$: 576.0872; found: 576.0874.

3,6-Di-(3'-ethylthio-4'-methylsuccinimidyl)chromen-2-one (9)

The reaction of 44 mg (0.12 mmol) of compound **6** in 3 mL dry DMSO with 27µL ethanethiol (0.36 mmol) was carried out according to the same protocol used to obtain compound **7**, giving compound **9** in 84% yield (49mg, 0.1 mmol). FTIR (KBr) (cm^{-1}) : , 1720, 1620, 600. ^{1}H-NMR ($CDCl_3$) : δ (ppm) 8.21 (s, 1H), 7.57 (d, *J* = 2.53 Hz, 1H), 7.39 (dd, *J* = 8.47 Hz, *J* = 2.67 Hz, 1H), 7.26 (d, *J* = 8.35 Hz, 1H), 3.84 (s, 1H), 3.12 (s, 1H), 3.07 (s, 1H, 2.83 (m, 4H), 1.93 (d, *J* = 7.08 Hz, 3H), 1.90 (d, *J* = 7.25 Hz, 3H), 1.30 (t, *J* = 7.28 Hz, 6H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 175.04, 172.32, 167.92, 156.34, 150.73, 137.21, 129.87, 128.65, 124.34, 118.03, 117.04,

38.53, 34.29, 26.02, 20.68, 14.27, 11.67. HRMS expected for $C_{23}H_{24}N_2O_6S_2$: 488.1076; found: 488.1072.

3,6-(4,4'-bis(methylmercapto-3-succinimidyl)benzophenone)chromen-2-one (10)

Compound **4** (42 mg, 0.13 mmol) and BMMB (34 mg, 0.13 mmol) were placed in a 10-mL round bottom flask. Chloroform (2.5 mL) was then added with stirring. To this suspension triethylamine (52 µL, 0.39 mmol) was then added and the reaction mixture was heated to reflux for 12 h. The mixture was cooled to room temperature. A 50-mL aliquot of water was then added and the solution was extracted 4 times with 20 mL dichloromethane. The organic layers were combined, dried over $MgSO_4$ and removed under reduced pressure. The resulting product was then purified by flash chromatography (20:80 EtOAc:hexane). Compound **10** was thus obtained as a beige solid in 78% yield (61 mg, 0.10 mmol). mp: >220 °C (dec). FTIR (KBr) (cm^{-1}) : 1720, 1654, 1605, 602. ^{1}H-NMR ($CDCl_3$) : δ (ppm) 7.81 (d, *J* = 8.0 Hz, 4H), 7.73 (d, *J* = 7.9 Hz, 4H), 7.60 (d, *J* = 9.4 Hz, 1H), 7.52 (s, 1H), 7.45 (d, *J* = 9.6 Hz, 1H), 7.39 (s, 1H), 4.40 (d, *J* = 5.6 Hz, 1H), 3.96 (d, *J* = 5.5 Hz, 1H), 3.68 (s, 4H), 3.26 (q, *J* = 8.8 Hz, 2H), 2.65 (d, *J* = 5.6 Hz, 2H). ^{13}C-NMR ($CDCl_3$) : δ (ppm) 195.92, 176.77, 175.24, 159.32, 148.90, 147.76, 143.07, 136.90, 136.51, 130.96, 130.25, 129.88, 129.38, 127.62, 127.08, 121.77, 117.69, 105.43, 36.70, 35.34, 28.47. HRMS expected for $C_{32}H_{22}N_2O_7S_2$: 611.0925; found : 611.0927.

6-Nitro-3-*p*-nitrophenylchromen-2-one (11)

2-Hydroxy-5-nitrobenzaldehyde (1.00 g, 6.00 mmol) and *p*-nitrophenylacetic acid (1.08 g, 6.00 mmol) were placed in a dry 25-mL round bottom flask. Acetic anhydride (11.2 mL, 120 mmol) was then added with stirring. To this suspension sodium hydride (240 mg, 6.00 mmol) was then

added in small aliquots (60% oil suspension) and the reaction mixture was stirred for 3 h at room temperature. The resulting beige powder was collected by filtration and washed with diethyl ether, then dried under vacuum to give compound **11** in 95 % yield (1.77 g, 5.67 mmol). mp : 250 °C (dec). FTIR (KBr) (cm^{-1}) : 1620, 1480, 1750, 1540, 1350. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 8.80 (d, *J* = 2.6 Hz, 1H), 8.62 (s, 1H), 8.47 (dd, *J* = 9.1 Hz, *J* = 2.7 Hz, 1H), 8.37 (dd, *J* = 8.9 Hz, *J* = 2.0 Hz, 1H), 8.02 (dd, *J* = 8.9 Hz, *J* = 2.0 Hz, 2H), 7.71 (d, *J* = 9.1 Hz, 1H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 159.43, 157.87, 148.46, 144.68, 142.30, 141.50, 130.85, 127.92, 125.77, 124.51, 120.53, 118.64. HRMS : expected, 312.0382; found, 312.0391. Elemental analysis for $C_{15}H_8N_2O_6$: expected : C, 57.70; H, 2.58; N, 8.97; found : C, 57.37; H, 2.62; N, 8.78.

6-Amino-3-*p*-aminophenylchromen-2-one (12)

Following the same protocol used to obtain diamine **2**, palladium on carbon (10% water) (0.322 g, 1/10th of the mass of **11**) and a solution of sodium borohydride (1.21 g, 32.9 mmol) in water (22.6 mL) were used to reduce a suspension of compound **11** (4.03 g, 12.9 mmol) in methanol (775 mL). In this way, compound **12** (2.04 g, 8.1 mmol) was obtained as a yellow solid in 63 % yield. FTIR (KBr) (cm^{-1}) : 3180, 3120, 1700, 1610, 850. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 7.84 (s, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.08 (d, *J* = 8.6 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 1H), 6.77 (s, 1H), 6.60 (d, *J* = 7.8 Hz, 2H), 5.32 (ds, 3H), 5.15 (ds, 3H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 163.34, 150.20, 146.43, 145.43, 137.99, 130.38, 127.88, 123.18, 11.33, 118.85, 116.94, 114.39, 111.04. HRMS : expected , 252.0899; found, 252.0898. Elemental analysis for $C_{15}H_{12}N_2O_2$: expected : C, 71.42; H, 4.79; N, 11.10; found : C, 71.30; H, 4.58; N, 10.92.

6-Maleimidyl-3-*p*-maleimidylphenylchromen-2-one (13)

The protocol used to obtain compound **4** was followed, using diamine **12** (400 mg, 1.59 mmol) and maleic anhydride (777 mg, 4.77 mmol) in 15 mL chloroform and then 15 mL acetic anhydride (0.16 mol) and 130 mg sodium acetate (1.59 mmol). In this way, **13** was obtained as a beige solid and dried over vacuum to give **13** in 20 % yield (131 mg, 0.32 mmol). mp : >250 °C (dec). FTIR (KBr) (cm^{-1}) : 2923, 1721, 1615, 1520, 1347, 853. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 8.36 (s, 1H), 7.87 (dd, *J* = 8.9 Hz, *J* = 2.0 Hz, 2H), 7.85 (d, *J* = 8.6 Hz, 1H), 7.60 (dd, *J* = 8.6 Hz, *J* = 2.6 Hz, 1H), 7.59 (d, *J* = 2.6 Hz, 1H), 7.45 (dd, *J* = 8.9 Hz, *J* = 2.0 Hz, 2H), 7.24 (d, 4H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 169.94, 159.60, 152.04, 141.00, 134.90, 133.82, 131.96, 130.50, 130.11, 129.25, 127.88, 126.95, 126.50, 119.77, 117.40, 104.30. HRMS : expected, 412.0695; found, 412.0695. Elemental analysis for $C_{23}H_{12}N_2O_6$: expected: C, 66.99; H, 2.93; N, 6.79; found: C, 66.13; H, 2.84; N, 6.64.

6-(3'-Ethylthiosuccinimidyl)-3-*p*-(3'-ethylthiosuccinimidyl)phenylchromen-2-one (14)

To a solution of compound **13** (20 mg, 0.049 mmol) in 2.5 mL of dry DMSO was added 110 µL of ethanethiol (0.147 mmol) and the solution was allowed to stir overnight at room temperature. A 100-mL aliquot of water was then added and the solution was extracted 4 times with 20 mL dichloromethane. The organic layers were combined, dried over $MgSO_4$ and removed under reduced pressure, apart from traces of residual DMSO, giving compound **14** in 89 % yield (22 mg, 0.044 mmol). FTIR (KBr) (cm^{-1}) : 2950, 1713, 1602, 835, 756. ^{1}H-NMR (DMSO–d_6) : δ (ppm 8.39 (s, 1H), 7.86 (d, *J* = 8.5 Hz, 2H), 7.74 (d, *J* = 2.5 Hz, 1H), 7.60 (d, *J* = 9.1 Hz, 1H), 7.55 (dd, *J* = 8.9 Hz, *J* = 2.5 Hz, 1H), 7.38 (d, *J* = 8.6 Hz, 2H), 4.17 (m, 2H), 3.42 (dd, *J* = 8.2 Hz, *J* = 2.0 Hz, 1H), 3.38 (dd, *J* = 8.2 Hz, *J* = 2.0 Hz, 1H), 2.84 (m, 4H), 2.76 (d, *J* = 5.6 Hz,

1H), 2.71 (d, *J* = 5.5 Hz, 1H), 1.25 (t, *J* = 7.3 Hz, 6H). ^{13}C-NMR (DMSO–d_6) : δ (ppm) 176.96, 175.33, 160.45, 153.44, 141.38, 135.52, 133.56, 131.38, 130.26, 129.42, 127.89, 127.79, 120.76, 117.79, 37.26, 25.82, 20.49, 19.31, 15.31. HRMS expected for $C_{27}H_{24}O_6S_2$: 537.1168; found: 537.1164.

1,5-Dimaleimidylnaphthalene (15)

The protocol used to obtain compound **4** was followed, using a suspension of 300 mg of 1,5-diaminonaphtalene (1.90 mmol) in 20 mL of dry chloroform, 560 mg (5.70 mmol) maleic anhydride, and then 15 mL acetic anhydride and 59 mg (0.70 mmol) of sodium acetate. Compound **15** was thus obtained as a yellow solid in 71% yield (428 mg, 1.35 mmol). mp >230 °C (dec). FTIR (KBr) (cm^{-1}) : 3070, 1705. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 7.87 (d, *J* = 7.80 Hz, 1H), 7.70 (t, *J* = 8.49 Hz, 1H), 7.62 (d, *J* = 7.38 Hz, 1H), 7.32 (s, 4H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 170.03, 134.61, 130.70, 128.24, 127.62, 126.31, 123.83. HRMS expected: 318.0641; found : 318.0641. Elemental analysis for $C_{18}H_{10}N_2O_4$ expected: C, 67.93; H, 3.17; N, 8.80; found: C, 67.05; H, 3.13; N, 8.62.

1,5-Di(3'-ethylthiosuccinimidyl)naphthalene (16)

The same protocol used to obtain **14** was followed, using 38 mg of compound **15** (0.12 mmol) in 3 mL anhydrous DMSO (3 mL) with 27 μL of ethanethiol (0.36 mmol) to give compound **16** in 87 % yield (46 mg, 0.10 mmol). FTIR (KBr) (cm^{-1}) : 3000, 1620, 600. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 8.00 (d, *J* = 8.16 Hz, 1H), 7.78 (t, *J* = 7.82 Hz, 1H), 7.75 (m, 2H), 7.57 (d, *J* = 8.04 Hz, 1H), 7.51 (d, *J* = 8.02 Hz, 2H), 4.42 (d, *J* = 5.20 Hz, 1H), 4.22 (d, *J* = 5.00 Hz, 1H), 3.64 (m, 1H), 3.42 (m, 1H), 3.34 (m, 6H), 1.89 (t, 6H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 175.95, 174.40,

162.92, 129.81, 129.56, 128.99, 128.84, 128.35, 127.06, 126.63, 126.31, 125.82, 124.01, 122.48, 36.43, 35.91, 24.70, 24.40, 13.86. HRMS expected for $C_{22}H_{22}N_2O_4S_2$: 442.1021; found: 442.1023.

3,6-Dinitro-1,8-naphthalic anhydride (17)[3]

Sulfuric acid (40.0 mL, 0.75 mol) was placed in a 100-mL flask to which was added 1,8-naphtalic anhydride (10.0g, 50.5 mmol). The solution was cooled to 5 °C in an ice bath. Nitric acid (10mL, 0.22 mol) was added drop by drop, taking care to not allow the temperature to exceed 20 °C. The solution was then heated to 60 °C for 90 min and then cooled again to 4 °C for 24 h to induce precipitation. The resulting precipitate was removed by filtration and washed with cold glacial acetic acid. The solid was then washed with toluene (3 × 50 mL) that was subsequently removed by rotary evaporation, repeating this process two more times to remove trace acetic acid, giving **17** as a beige powder (10.31g, 37.8 mmol) in 72 % yield. mp 208-210 °C (lit[3] 208 °C. FTIR (KBr) (cm^{-1}) : 3098, 2897, 1707, 1617, 1552, 1336, 1260. ^{1}H-NMR (acetone-d_6) : δ (ppm) 9.80 (d, *J* = 2.23 Hz, 2H), 9.05 (d, *J* = 2.13 Hz, 2H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 159.75, 148.14, 134.62, 133.28, 131.62, 128.06, 123.07. HRMS expected : 288.0019; found : 288.0014.

***N*-(3,6-Dinitro-1,8-naphthalyl)-L-aspartic acid dimethyl ester (18)**

Compound **17** (300 mg, 1.06 mmol) and L-aspartic acid dimethyl ester hydrochloride (217 mg, 1.06 mmol) were added to a 25-mL flask containing 10 mL of dry acetonitrile. Triethylamine (180 µL, 1.27 mmol) was added drop by drop to give a violet solution that was heated to 90 °C for 60 h. After cooling the solution to room temperature, the solvent was removed through

rotary evaporation and the solid obtained was purified by flash chromatography (70:30 hexane :EtOAc). The solvent was evaporated to give compound **18** as a beige powder (229 mg, 0.53 mmol) in 50 % yield. mp : 128-129 °C. FTIR (KBr) (cm^{-1}) : 3083, 2954, 1740, 1678, 1536, 1319, 748. ^{1}H-NMR ($CDCl_3$) : δ (ppm) 9.44 (d, *J* = 2.08 Hz, 2H), 9.39 (d, *J* = 2.07 Hz, 2H), 6.17 (dd, *J* = 7.88 Hz, *J* = 6.29 Hz, 1H), 3.72 (s, 3H), 3.63 (s, 3H), 3.44 (dd, *J* = 16.84 Hz, *J* = 6.26 Hz, 1H), 3.01 (dd, *J* = 16.84, *J* = 7.94 Hz, 1H). ^{13}C-NMR ($CDCl_3$) : δ (ppm) 170.53, 168.37, 160.99, 147.46, 131.97, 130.90, 130.80, 127.58, 124.54, 52.98, 52.00, 50.12, 33.35. HRMS expected for $C_{18}H_{14}N_3O_{10}$: 432.0679; found : 432.0688.. α_D= -33° (c = 1, $CHCl_3$).

***N*-(3,6-Diamino-1,8-naphthalyl)-L-aspartic acid dimethyl ester (19)**

Compound **18** (220 mg, 0.51 mmol) was placed in a 50-mL flask and 20 mL of THF and palladium on carbon (10% wet) (22 mg, 1/10th the mass of **18**) were added. The reaction mixture was then placed in a hydrogenation reactor and left under 150 psi pressure for 24 h. The mixture was then filtered through Celite and the filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography (70:30 EtOAc:$CHCl_3$). The solvent was then removed by rotary evaporation to give compound **19** (92.5 mg, 0.25 mmol) as an orange powder in 49 % yield. mp : 175-177 °C. FTIR (KBr) (cm^{-1}) : 3380, 1748, 1625, 1316, 797. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 7.59 (d, *J* = 1.90 Hz, 2H), 6.97 (d, *J* = 1.90 Hz, 2H), 6.02 (dd, *J* = 8.00 Hz, *J* = 5.43 Hz, 1H), 5.74 (s, 4H), 3.61 (s, 6H), 3.28 (dd, *J* = 16.49 Hz, *J* = 8.59 Hz, 1H), 2.85 (dd, *J* = 16.53 Hz, *J* = 5.00 Hz, 1H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 171.81, 170.66 , 164.56, 148.76, 136.60, 122.74, 118.43, 115.51, 111.18, 111.11, 53.50, 50.73, 49.52, 34.76. HRMS expected for $C_{18}H_{17}N_3O_6$: 371.1117; found : 371.1135. α_D= -150° (c = 0.1, DMSO).

***N*-(3,6-Dimaleimidyl-1,8-naphthalyl)-L-aspartic acid dimethyl ester (20)**

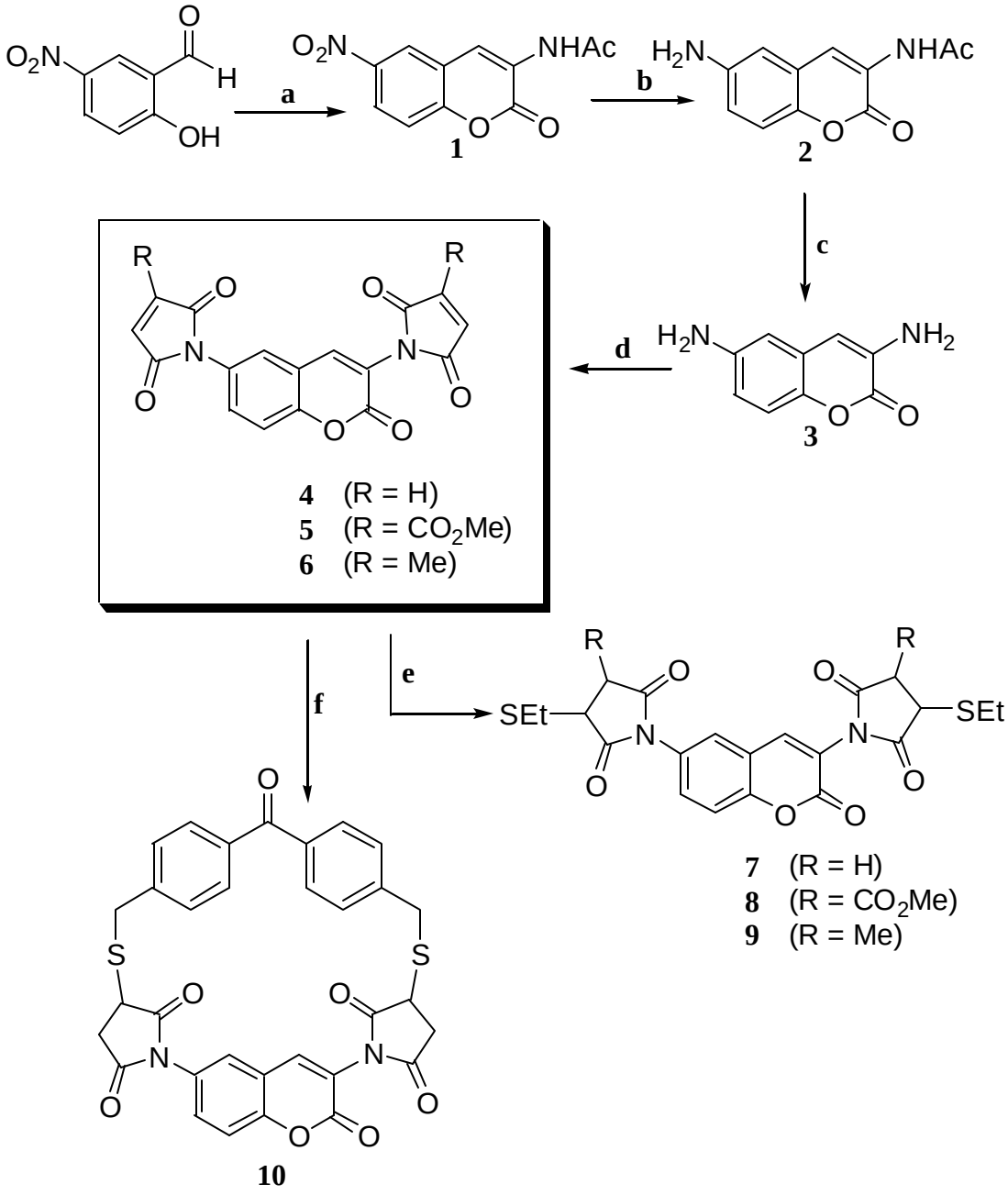
Compound **19** (200 mg, 0.54 mmol) and maleic anyhdride (159 mg, 1.62 mmol) were placed in a dry 50-mL round-bottom flask. Chloroform (15 mL) was added and the solution was heated to reflux for 24 h. The solution was then cooled to room temperature and filtered. The brown precipitate thus recovered was dried under reduced pressure. The solid was then placed in a 25-mL round-bottom flask to which sodium acetate (21 mg, 0.37 eq) and acetic anhydride (10 mL) were added. The solution was heated to 100 °C for 90 min. The solvent was then removed through evaporation and the resulting solid was dissolved in ethyl acetate. The solution was cooled to 4°C for 48 h and the recovered precipitate was washed with 25 mL of 80:20 water-DMSO and then 25 mL of water. The solid was then dissolved in a minimal volume of hot ethyl acetate and hexane was added to induce precipitation of the expected product (**20**) (53 mg, 0.10 mmol) as an orange powder in 19 % yield. mp >230 °C (dec). FTIR (KBr) (cm^{-1}) : 3102, 1717, 1669, 1413. ^{1}H-NMR (DMSO-d_6) : δ (ppm) 8.60 (d, *J* = 1.83 Hz, 2H), 8.58 (d, *J* = 1.86 Hz, 2H), 7.32 (s, 4H), 6.12 (dd, *J* = 7.96 Hz, *J* = 5.38 Hz, 1H), 3.64 (s, 3H), 3.63 (s, 3H), 3.32 (dd, *J* = 16.52 Hz, *J* = 8.60 Hz, 1H), 2.97 (dd, *J* = 16.53 Hz, *J* = 5.03 Hz, 1H). ^{13}C-NMR (DMSO-d_6) : δ (ppm) 171.77, 170.63, 170.33, 163.45, 136.08, 132.70, 132.35, 132.16, 130.80, 125.85, 123.47, 53.72, 52.83, 50.16, 34.46. HRMS expected: 532.0992; found: 532.0980. α_D = -85° (c = 0.1, DMSO).

***N*-(3,6-Di(3'-ethylthiosuccinimidyl)-1,8-naphthalyl)-L-aspartic acid dimethyl ester (21)**

The reaction of 26 mg (0.49 mmol) of compound **20** in 2.5 mL dry DMSO with 110 µL ethanethiol (0.147 mmol) was carried out according to the same protocol used to obtain compound **7**, giving compound **21** in 89% yield (29 mg, 0.044 mmol). FTIR (KBr) (cm^{-1}) :

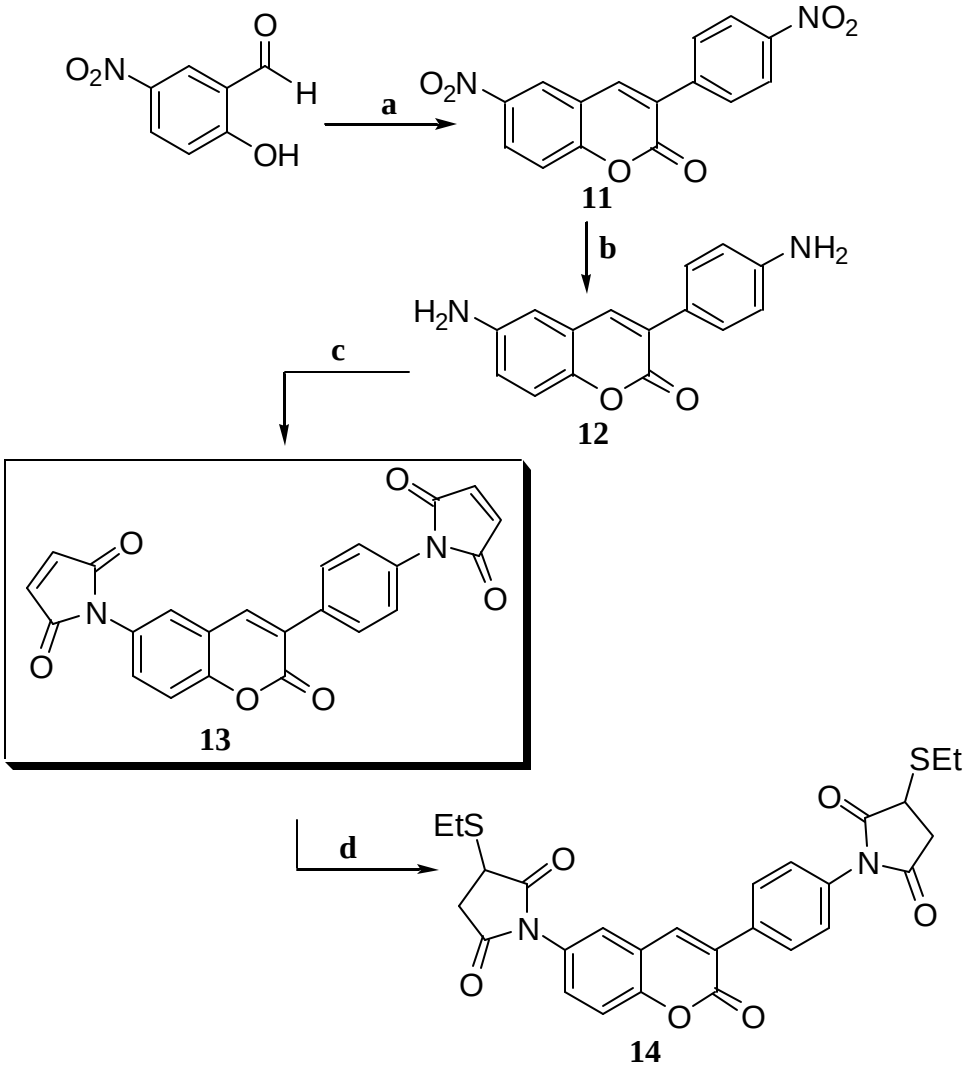
3104, 1715, 1671, 1528, 756. ^{1}H-NMR (DMSO–d_6) : δ (ppm) 8.59 (d, *J* = 1.82 Hz, 2H), 8.52 (d, *J* = 1.78 Hz, 2H), 6.08 (dd, *J* = 7.86, *J* = 5.29 Hz, 1H), 4.20 (q, *J* = 8.66 Hz, 2H), 3.61 (m, 10 H), 3.41 (dd, *J* = 16.46, *J* = 8.56 Hz, 1H), 2.88 (m, 5H), 1.25 (t, *J* = 8.27 Hz, 6H). ^{13}C-NMR (DMSO–d_6) : δ (ppm) 175.86, 173.73, 170.26, 169.15, 168.80, 161.87, 134.58, 131.96, 131.20, 130.68, 129.30, 127.72, 127.34, 126.98, 124.74, 124.46, 121.98, 121.73, 52.18, 51.32, 48.64, 32.93, 24.44, 18.98, 17.78, 13.84. HRMS expected for $C_{30}H_{29}O_{10}S_2$: 655.1294; found: 655.1290.

Scheme 1



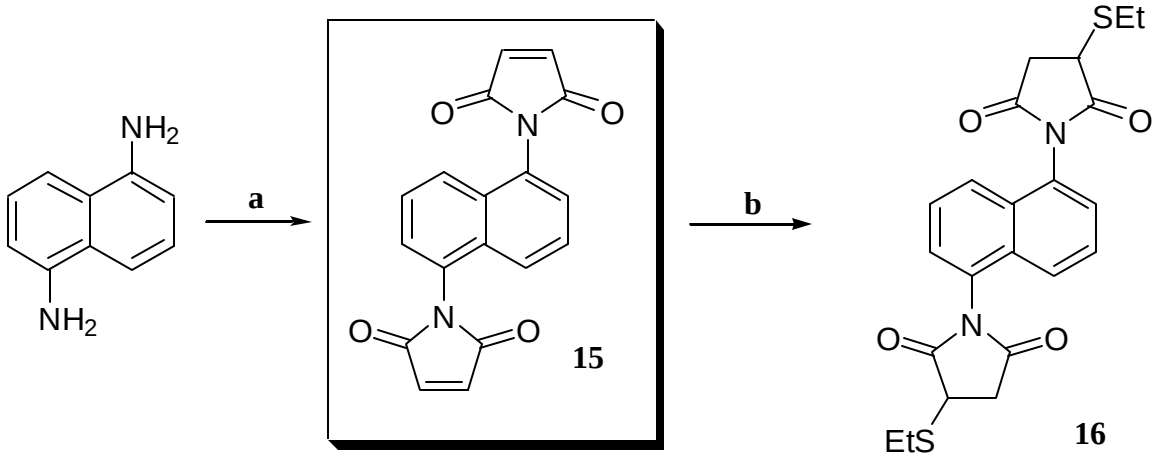
(a) *N*-acetylglycine, NaH, Ac_2O, rt, 61%; (b) Pd/C (10% H_2O), $NaBH_4$, MeOH, 57%; (c) EtOH, HCl, reflux, 65%; (d) (i) maleic anhydride, $CHCl_3$, reflux, (ii) Ac_2O, AcONa, 100 °C, 20% (2 steps); (e) EtSH, $CHCl_3$ or DMSO, rt, 83-85%; (f) BMMB, NEt_3, $CHCl_3$, reflux, 78%.

Scheme 2



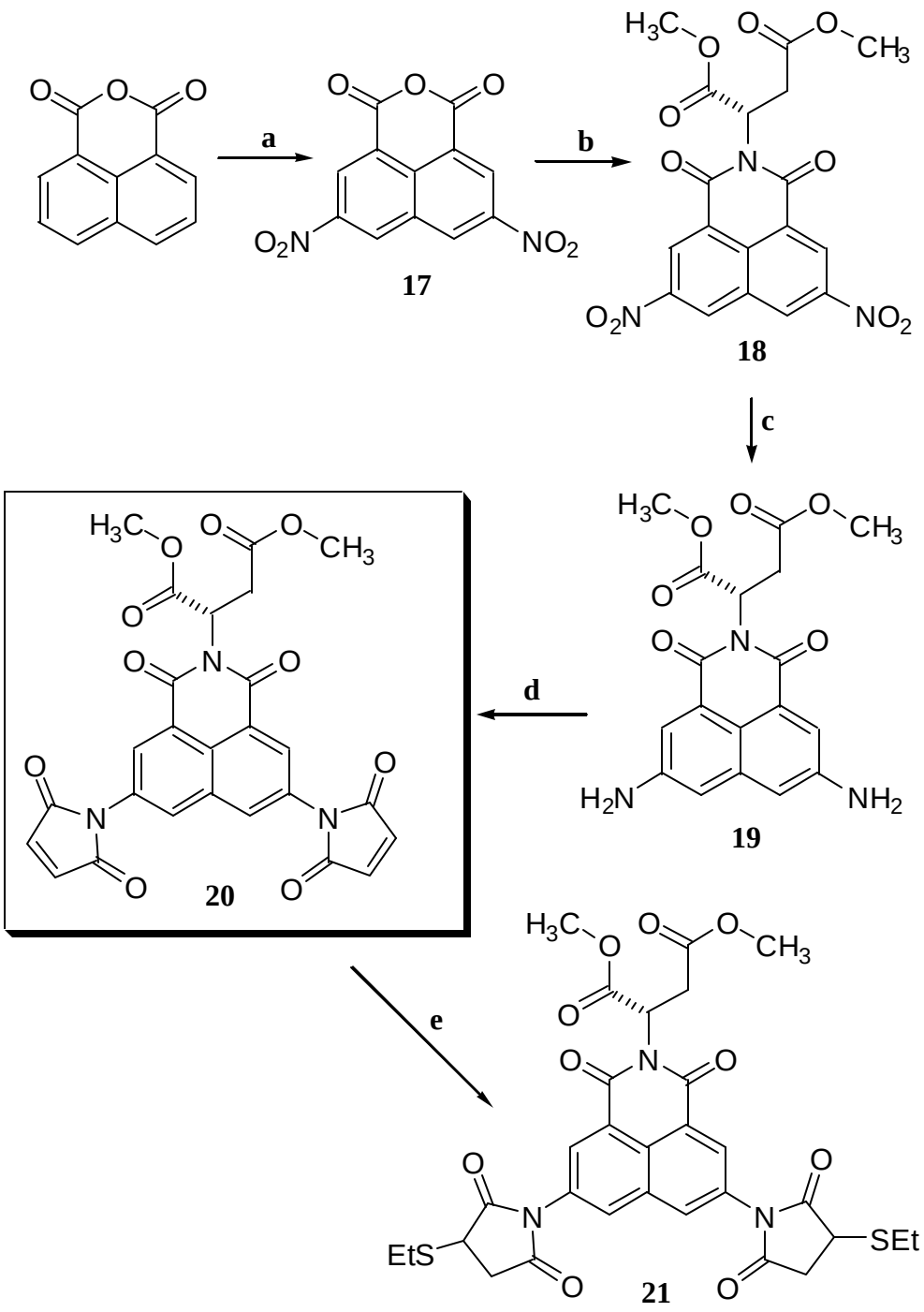
(a) 4-nitrophenylacetic acid, Ac_2O, NaH, rt, 95%; (b) Pd/C (10% H_2O), $NaBH_4$, MeOH, 63%; (c) (i) maleic anhydride, $CHCl_3$, reflux, (ii) Ac_2O, AcONa, 100°C, 20% (2 steps) (d) EtSH, DMSO, rt, 89%.

Scheme 3



(a) (i) maleic anhydride, $CHCl_3$, reflux, (ii) Ac_2O, AcONa, 100 °C, 75% (2 steps) (b) EtSH, DMSO, rt, 87%.

Scheme 4



(a) H_2SO_4, HNO_3, 10-20 °C, 72% (b) CH_3CN, TEA, L-aspartic acid dimethyl ester•HCl, 90 °C, 45% (c) H_2/Pd/C, 150 psi, THF, 49% (d) (i) maleic anhydride,$CHCl_3$, reflux, (ii) Ac_2O, AcONa, 100 °C, 19% (2 steps). (e) EtSH, DMSO, rt, 89%.

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