Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# A Simple FRET-Based Modular Design for Diagnostic Probes

## Supporting Information

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| Synthetic Schemes and Experimental Procedures | S2-S17 |
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**General methods.** All reactions requiring anhydrous conditions were performed under an Argon atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either A.R. grade or purified by standard techniques. Thin layer chromatography (TLC): silica gel plates Merck 60 $F_{254}$: compounds were visualized by irradiation with UV light. Flash chromatography (FC): silica gel Merck 60 (particle size 0.040-0.063 mm), eluent given in parentheses. $^{1}$H-NMR spectra were measured using Bruker Avance operated at 200MHz or 400MHz as mentioned. $^{13}$C-NMR spectra were measured using Bruker Avance operated at 50MHz or 100MHz as mentioned. The chemical shifts are expressed in δ relative to TMS (δ = 0 ppm) and coupling constants *J* in Hz. The spectra were recorded in $CDCl_3$ as solvent at room temperature unless stated otherwise. All general reagents, including salts and solvents, were purchased from Sigma-Aldrich.

**Abbreviations. AcOH**- Acetic acid, **ACN**- Acetonitrile, **DBTL**- Dibutyltin dilaurate, **DCM**- Dichloromethane, **DMAP**- 4-Dimethylaminopyridine, **DMF**- N,N'-Dimethylformamide, **$Et_2O$**- Diethyl ether, **EtOAc**- Ethylacetate, **Hex**- n-Hexanes, **MeOH**- Methanol, **PTSA**- *p*-Toluene sulfonic acid, **THF**- Tetrahydrofurane, TBSCl- Chloro trimethylsilane, **TFA-** Trifluoroacetic acid, **$Et_3N$**- Triethylamine, **TBSCl**- t-Butyldimethylsillyl chloride, **DR1-** disperse red 1, **Boc-** *t*-butoxycarbonyl, **NHS-** N-hydroxysuccinimide, **DIPEA**- N,N-Diisopropylethylamine, **EtOH**- Ethyl alcohol, **NaOAc**- Sodium acetate, **$Ac_2O$** – Acetic anhydride, **DCC**- N,N'-Dicyclohexylcarbodiimide.

Compound **3a**

Compound **3**[1] (55 mg, 0.13 mmol) was dissolved in 2 ml of dry THF and cooled to -20°C. Then DIPEA (181µL, 1.04 mmol) was added, followed by the addition of *p*-nitrophenyl-chloroformate (158 mg, 0.78 mmol) dissolved in 2 ml of dry THF. Then a catalytic amount of Pyridine was added and the reaction was stirred at -20°C for 3 h. The reaction was monitored by TLC (EtOAc/Hex 50:50). Upon completion, the reaction mixture was diluted with EtOAc and washed with saturated aqueous solutions of $NH_4Cl$. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 50:50) to afford compound **3a** (35 mg, 36%). Compound **3a** was immediately used in the next step without further characterization.

Compound **2**

Compound **3a** (10 mg, 0.013 mmol) was dissolved in 1 ml DMF. Then compound **3b** (24 mg, 0.029 mmol) dissolved in 1 ml DMF was added followed by the addition of $Et_3N$ (5µL, 0.033 mmol). The reaction mixture was stirred at room temperature for 72h and monitored by RP-HPLC (10-90% ACN in $H_20$, 20 minutes). Upon Completion, the solvent was removed under reduced pressure and the crude product was purified by preparative RP-HPLC (10-90% ACN in $H_20$, 20 minutes) to afford compound **2** (11 mg, 44%) as a blue solid.

$^1$H NMR (200 MHz, DMSO): δ = 10.23 (1H, s), 9.77 (1H, s), 9.21 (1H, s), 8.41-8.28 (4H, m), 7.816 (4H, s), 7.67-7.55 (7H, m), 7.37-7.55 (11H, m), 6.89 (2H, m), 6.56-6.38 (2H, m), 6.26-6.22 (4H, m), 5.01-4.98 (6H, m), 4.05-4.01 (8H, m), 3.72 (2H, s), 3.34 (8H, m), 2.76 (4H, m), 1.65 (36H, m), 1.22 (6H, m). $^{13}$C NMR (200MHz, DMSO): δ = 176.23, 173.74, 169.09, 154.36, 152.71, 148.31, 146.83, 142.19, 138.97, 134.35, 132.53, 131.10, 127.38, 126.22, 124.75, 122.91, 115.63, 114.89, 105.72, 67.93, 61.56, 56.08, 44.78, 42.62, 37.96, 36.71, 28.31, 27.17, 26.77, 24.52, 14.23. MS (ESI-): *m/z* calc. for $C_{96}H_{112}N_{10}O_{21}S_4$: 935.8; found: 972.8 $[M-H+K]^-$

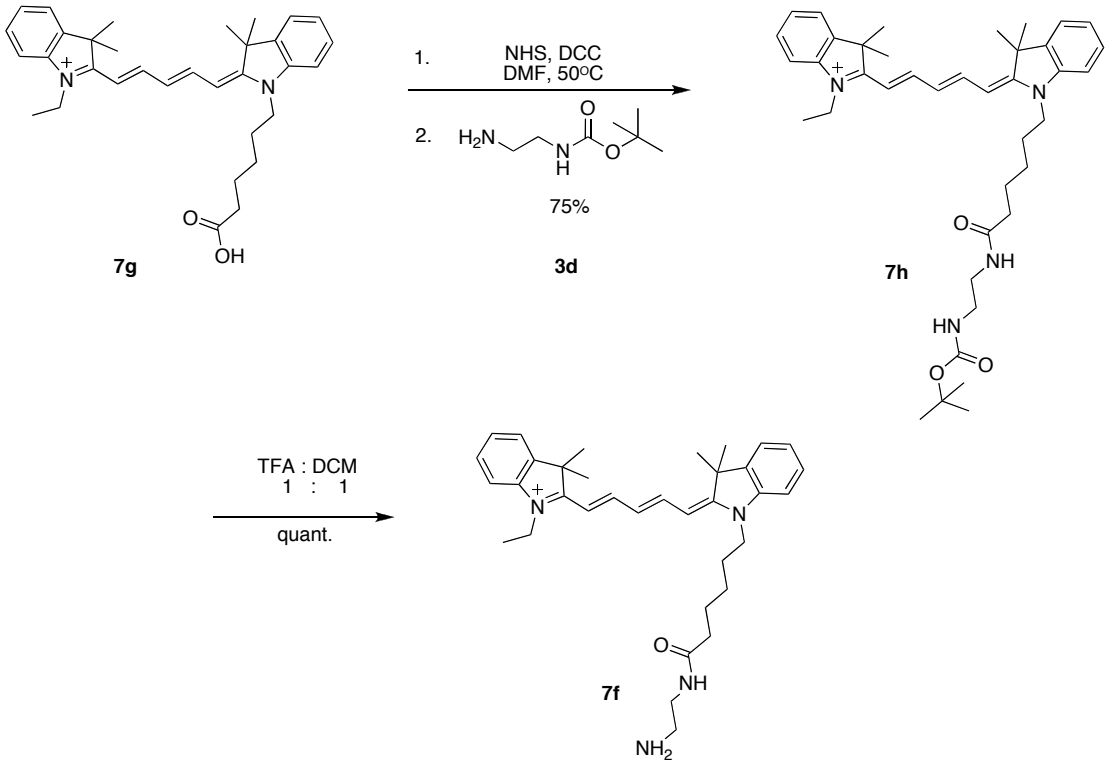


***Figure 1***: Chemical synthesis of compound **3b**.

Compound **3e**

Compound **3c**[2] ( 300 mg, 0.46 mmol) was dissolved in 10 ml DMF. Then DCC (142 mg, 0.69 mmol) was added followed by the addition of NHS (79 mg, 0.69 mmol). The mixture was heated to 50°C and stirred for 2h. Then compound **3d**[3] (110 mg, 0.69 mmol) was added and the mixture was stirred ON at room temperature. The reaction was monitored by TLC (DCM/MeOH 70:30). Upon completion the solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (DCM/MeOH 70:30) to afford compound **3e** (271 mg , 74%) as a blue solid.

$^{1}$H NMR (200 MHz, DMSO): δ = 8.43 (2H, t, *J* = 13Hz), 7.81 (2H, s), 7.66 (2H, d, *J* = 8Hz), 7.34 (2H, d, *J* = 8Hz), 6.64 (1H, t, *J* = 12Hz), 6.34 (2H, d, *J* = 13Hz), 4.14-4.09 (4H, m), 3.03-2.91 (4H, m), 2.06 (2H, t, *J* = 6.2Hz), 1.68 (12H, s), 1.59-1.52 (4H, m), 1.38 (2H, m), 1.35 (9H, s), 1.29-1.11 (3H, m). $^{13}$C NMR (200MHz, DMSO): δ = 173.79, 172.98, 170.63, 156.11, 154.94, 145.52, 142.19, 141.73, 140.82, 127.28, 120.42, 110.22, 108.57, 103.75, 103.48, 78.05, 49.25, 44.13, 35.48, 28.55, 27.42, 27.32, 26.16, 25.26, 14.37, 12.35. MS (ESI-): *m/z* calc. for $C_{40}H_{54}N_4O_9S_2$: 799.3; found: 798.3 $[M-H]^-$.

Compound **3b**

Compound **3e** (21mg, 0.029 mmol) was treated with a mixture of TFA:DCM (1:1) for 5 minutes, which was then evaporated to afford compound **3b** in quantative yield**.** The amine was immediately used in the next step without further purification

Compound **6c**

Compound **6b**[4] (5.28 gr, 29.64 mmol), was dissolved in cold (0°C) aqueous NaOH (23 ml, 12% solution). Then aqueous formaldehyde (19.7 ml, 37% solution) was added and the reaction mixture was stirred for 72h at 55°C. The reaction was monitored by TLC (EtOAc/Hex 50:50). Upon completion the reaction mixture was diluted with EtOAc and washed with saturated aqueous solution of $NH_4Cl$ followed by brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was dissolved in a minimum of hot acetone, cooled, and filtered to afford compound **6c** (2.11 gr, 30%) as a white solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ = 7.76 (2H, s), 5.97 (1H, m), 5.41-5.21 (2H, m), 4.77 (4H, s), 4.76 (2H, d, *J* = 5.6Hz). $^{13}$C NMR (200MHz, $CD_3OD$): δ = 166.3, 157.8, 132.9, 128.2, 126.9, 120.6, 116.8, 64.8, 60.1. MS (ESI-): *m/z* calc. for $C_{12}H_{14}O_5$: 238.1; found: 237.1 $[M-H]^-$.

Compound **6d**

Compound **6c** (835 mg, 3.5 mmol) was dissolved in minimal amount of DMF and cooled to 0° C. Imidazole (477.5 mg, 7 mmol) and TBSCl (1.06 gr, 7 mmol) were added. The reaction was allowed to warm to room temperature and was stirred for additional 1h. The reaction was monitored by TLC (EtOAc/Hex 5:95). Upon completion the reaction mixture was diluted with diethyl ether and washed with saturated aqueous solution of $NH_4Cl$ followed by brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOac/Hex 5:95) to afford compound **6d** (1.22 gr, 75%) as colorless oil.

$^1$H NMR (400 MHz, $CDCl_3$): δ = 8.79 (1H, s), 7.86 (2H, s), 6.04 (1H, m), 5.39-5.2 (2H, m), 4.85 (4H, s), 4.75 (2H, d, *J* = 5.6Hz), 0.93 (18H, s), 0.13 (12H, s). $^{13}$C NMR (400MHz, $CDCl_3$): 166.7, 158.3, 133.2, 128.4, 126.6, 121.9, 118.3, 65.7, 63.4, 26.5, 18.9, -4.76. MS (ESI+): *m/z* calc. for $C_{24}H_{42}O_5Si_2$: 466.2; found: 489.2 $[M+Na]^+$.

Compound **6f**

Compound **6d** (433 mg, 0.95 mmol) was dissolved in 2 ml DMF and cooled to 0° C. $K_2CO_3$ (222.3 mg, 1.6 mmol) was added and the solution stirred at 0°C for 10 min, before compound **6e**[5] (553.5 gr, 1.6 mmol) was added. The reaction mixture was stirred for 12h at room temperature and monitored by TLC (EtOAc/Hex 5:95). After completion, the reaction mixture was diluted with diethyl ether and washed with saturated aqueous solution of $NH_4Cl$ followed by brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOac/Hex 5:95) to afford compound **6f** (537 gr, 83%) as yellow oil.

$^1$H NMR (200 MHz, $CDCl_3$): δ = 8.11 (2H, s), 7.85 (2H. d, *J* = 8Hz), 7.41 (2H, d, *J* = 8Hz), 6.04 (1H, m), 5.49-5.29 (2H, m), 4.96 (2H, s), 4.82 (2H, d, *J* = 5.6Hz), 4.7 (4H, s), 1.35 (12H, s), 0.91 (18H, s), 0.07 (12H, s). $^{13}$C NMR (200MHz, $CDCl_3$): δ =165.8, 157.1, 139.8, 135, 134.5, 132.2, 129.1, 126.9, 126, 117.6, 83.7, 76.1, 65.1, 60, 25.8, 24.7, 18.2, -5.4. MS (CI+): *m/z* calc. for $C_{37}H_{59}BO_7Si_2$: 682.39; found: 683 $[M+H]^+$.

Compound **6g**

Compound **6f** (463 mg, 0.67 mmol) was dissolved in MeOH, and catalytic amount of *p*-TsOH was added. The reaction mixture was stirred for 15 min in RT, and monitored by TLC (EtOAc/Hex 50:50). After completion, the reaction mixture was diluted with EtOAc and washed with saturated aqueous solution of $NaHCO_3$. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOac/Hex 40:60) to afford compound **6g** (185 mg, 60%) as colorless oil.

$^1$H NMR (200 MHz, $CDCl_3$): δ = 8 (2H, s), 7.8 (2H. d, *J* = 8Hz), 7.37 (2H, d, *J* = 8Hz), 5.96 (1H, m), 5.41-5.21 (2H, m), 4.92 (2H, s), 4.75 (2H, d, *J* = 5.6Hz), 4.63 (4H, s), 1.33 (12H, s). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 165.8, 158.352, 139.3, 135, 134.5, 132, 130, 127, 126, 118, 83.8, 76.6, 65.6, 60, 24.7. MS (ESI-): *m/z* calc. for $C_{25}H_{31}BO_7$: 454.2; found: 453.2 $[M-H]^-$.

Compound **6h**

Compound **6g** (181 mg, 0.399 mmol) was dissolved in 4 ml of dry THF. Then $Et_3N$ (276 μL, 1.995 mmol) and catalytic amount of DMAP were added and the mixture

was added dropwise to a solution of *p*-nitrophenyl chloroformate (241.3 mg, 1.197 mmol) dissolved in 2 ml dry THF at 0°C. The reaction was stirred for 1h in room temperature and monitored by TLC (EtOAc/Hex 30:70). After completion, the reaction mixture was diluted with EtOAc and washed with saturated aqueous solutions of $NH_4Cl$ and $NaHCO_3$. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOac/Hex 20:80) to afford compound **6h** (188 mg, 60%) as yellow oil.

$^1$H NMR (200 MHz, $CDCl_3$): δ = 8.26 (4H, d, *J* = 9.2Hz), 8.24 (2H, s), 7.87 (2H. d, *J* = 8Hz), 7.48 (2H, d, *J* = 8Hz), 7.33 (4H, d, *J* = 9.2Hz), 6.04 (1H, m), 5.47-5.29 (2H, m), 5.36 (4H, s), 5.14 (2H, s), 4.87 (2H, d, *J* = 5.6Hz), 1.36 (12H, s). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 165, 160.2, 155.2, 152.2, 145.3, 138.8, 135.1, 133.2, 131.7, 129, 127.8, 125.2, 121.7, 118.7, 84, 77.6, 66, 65.6, 24.7. MS (ESI+): *m/z* calc. for $C_{39}H_{37}BN_2O_{15}$: 784.2; found: 807.2 $[M+Na]^+$.

Compound **6i**

Compound **6h** (54 mg, 0.068 mmol) was dissolved in DCM**.** Then DR1 (21.63 mg, 0.068 mmol) and DMAP (8.3 mg, 0.068 mmol) were added. The reaction mixture was sttired for 2h and monitored by TLC (EtOAc/Hex 30:70). After completion, the reaction mixture was diluted with EtOAc and washed with saturated aqueous solution of $NH_4Cl$ followed by brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOac/Hex 30:70) to afford compound **6i** (33 mg, 50%) as red solid.

$^1$H NMR (200 MHz, $CDCl_3$): δ = 8.33-8.17 (6H, m), 7.93-7.81 (6H, m), 7.44 (2H, d, *J* = 8Hz), 7.29 (2H, d, *J* = 9.2Hz), 6.78 (2H, d, *J* = 9.2Hz), 6.02 (1H, m), 5.45-5.33 (2H, m), 5.29 (2H, s), 5.25 (2H, s), 5.05 (2H, s), 4.85 (2H, d, *J* = 6.2Hz), 4.35 (2H, m), 3.7 (3H, m), 3.52 (4H, m), 1.34 (12H, s). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 165.9, 158.2, 157.4, 155.5, 153.1, 151.8, 150.1, 144.8, 144.7, 136.7, 133.6, 132.1, 130.4, 128.1, 127.3, 127.1, 125.3, 125.1, 124.2, 122.8, 122.5, 120.9, 118.2, 111.7, 83, 136.7, 66.7, 67, 65.8, 55.3, 44.6, 25, 12.9. MS (ESI+): *m/z* calc. for $C_{49}H_{50}BN_5O_{15}$: 959.3; found: 982.3 $[M+Na]^+$.

Compound **6j**

Compound **6** (29.6 mg, 0.058 mmol), was dissolved in DMF, followed by the addition of $Et_3N$ (35 μL, 0.25 mmol) and compound **6i** (47 mg, 0.049 mmol). The reaction mixture was sttired for 2h and monitored by TLC (EtOAc/MeOH 95:5). After completion, the mixture was diluted with EtOAc and washed with saturated aqueous solution of $NH_4Cl$ followed by brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOac/MeOH 95:5) to afford compound **6j** (24 mg, 40%) as red solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ = 8.32 (2H, d, *J* = 8.76Hz), 8.08 (2H, s), 7.91 (2H, d, *J* = 8.76Hz), 7.86-7.8 (4H, m), 7.56-7.47 (4H, m), 7.41-7.39 (2H, m), 7.34 (1H, m), 7.05-6.99 (2H, m), 6.76 (2H, d, *J* = 8.96Hz), 6.63-6.59 (3H, m), 5.99 (1H, m), 5.4-5.25 (2H, m), 5.2 (2H, s), 5.18 (2H, s), 4.99 (2H, s), 4.79 (2H, d, *J* = 5.4Hz), 4.32 (2H, m), 3.68 (3H, m), 3.5-3.48 (4H, m), 3.4-3.29 (4H, m), 2.98 (3H, s), 2.81 (3H, s), 1.33 (12H, s). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 185.7, 174.2, 165.9, 158.4, 158.2, 156.9, 156.1, 155.5, 155.1, 151.8, 150.1, 147.9, 144.8, 136.7, 134.3, 133.6, 132.1, 130.4, 129.6, 128.1, 127.3, 127.1, 125.1, 124.2, 123.2, 122.8, 120.9, 118.2, 115.3, 111.7, 111.5, 110, 108.4, 100, 83, 71.4, 67, 66.7, 65.8, 60.3, 55.3, 49.4, 48.3, 44.6, 35, 34.9, 25, 12.9. MS (ESI+): *m/z* calc. for $C_{67}H_{67}BN_6O_{16}$: 1222.2; found: 1223.2 $[M+H]^+$.

Compound **4**

Compound **6j** (20 mg, 0.016 mmol) was dissolved in THF (0.5 ml). Then acetic acid (5 μL, 0.081 mmol ), $Bu_3SnH$ (26.3 μL, 0.097 mmol) and a catalytic amount of $Pd(PPh_3)_4$ was added. The reaction mixture was stirred for 15 minutes and monitored by TLC (EtOAc/MeOH/AcOH 94:5:1). Upon completion of the reaction, the solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (EtOac/MeOH/AcOH 94:5:1) to afford compound **4** (12.5 mg, 65%) as red solid.

$^1$H NMR (400 MHz, $CDCl_3$): δ = 8.32 (2H, d, *J* = 8.76Hz), 8.08 (2H, s), 7.91-7.8 (6H, m), 7.56-7.47 (4H, m), 7.41-7.39 (2H, m), 7.34 (1H, m), 7.05-6.99 (2H, m), 6.76 (2H, d, *J* = 8.96Hz), 6.63-6.59 (3H, m), 5.2 (2H, s), 5.18 (2H, s), 4.94 (2H, s), 4.32 (2H, m), 3.68 (3H, m), 3.5-3.48 (4H, m), 3.4-3.29 (4H, m), 2.98 (3H, s), 2.81 (3H, s), 1.33 (12H, s). $^{13}$C NMR (400MHz, $CDCl_3$): δ = 185.7, 174.2, 169.3, 159.1, 158.4, 156.9,

156.1, 155.5, 155.1, 151.8, 150.1, 147.9, 144.8, 136.7, 134.3, 133.6, 130.4, 129.6, 128.5, 127.3, 127.1, 125.6, 125.1, 124.2, 123.2, 120.9, 115.3, 111.7, 111.5, 110, 108.4, 155, 83.1, 71.4, 66.7, 65.8, 60.3, 55.3, 49.4, 48.3, 44.6, 35.1, 34.9, 24.7, 12.9. MS (ESI-): *m/z* calc. for $C_{64}H_{63}BN_6O_{16}$: 1182.5; found: 1182.5. $[M]^-$.

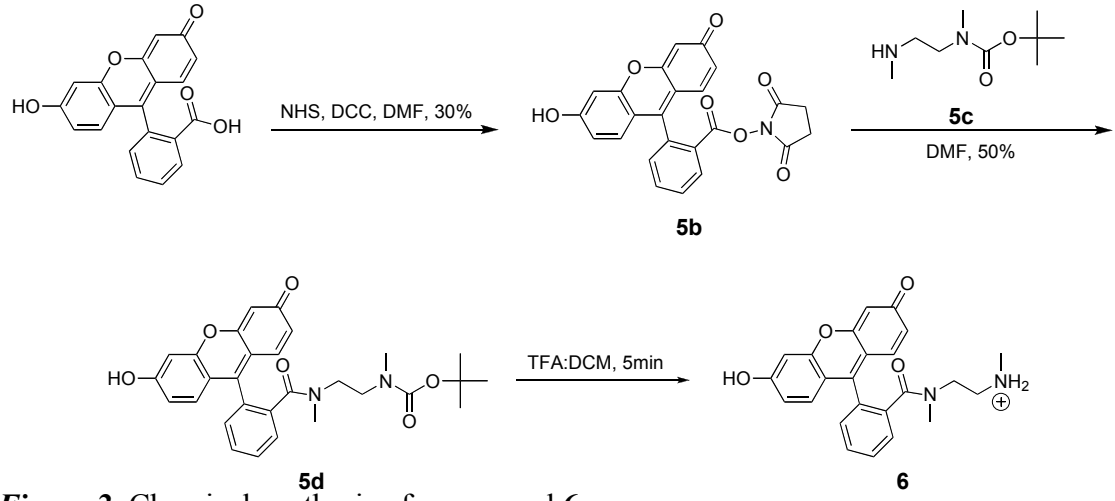


***Figure 2***: Chemical synthesis of compound **6**.

Compound **5d**

Compound **5b**[6] (200 mg, 0.46 mmol) was dissolved in 2 ml DMF and compound **5c**[7] (105 mmol, 0.55 mmol) was added**.** The reaction mixture was stirred for 12h at room temperature and monitored by TLC (EtOAc/MeOH 90:10). Upon completion of the reaction, the solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (EtOac/MeOH 90:10) to afford compound **5d** (196 mg, 84%) as orange solid.

$^1$H NMR (200MHz, $CDCl_3$): δ = 7.61 (4H, m), 7.34 (1H, m), 7.06-7.02 (2H, m), 6.69 (3H, m), 3.32-3.22 (4H, m), 2.83 (3H, s), 2.69 (3H, s), 1.39 (9H, s). $^{13}$C NMR (400MHz, $CDCl_3$): δ = 176, 169.6, 158.2, 156.8, 156.1, 153, 152.6, 136.7, 135.15, 132, 131.7, 131.1, 130.2, 128.4, 122.4, 116.5, 115.8, 114.8, 104.5, 80.4, 47.4, 45.8, 38.6, 35.4, 29.1. MS (ESI+): *m/z* calc. for $C_{29}H_{30}N_2O_6$: 502.2; found: 525.2 $[M+Na]^+$.

Compound **6**

Compound **5d** was treated with a mixture of TFA:DCM (1:1) for 5 minutes, which was then evaporated to afford amine **6** in quantative yield. The amine was immediately used in the next step without further purification.

Compound **7b**

Compound **7a**[8] (250 mg , 0.63 mmol) was dissolved in 3 ml DMF and cooled to 0° C. $K_2CO_3$ (174 mg, 1.26 mmol) was added and the mixture was stirred at 0°C for 10 min, before compound **6e**[5] (217 mg, 0.63 mmol) was added. The reaction mixture was stirred for 1h at room temperature and monitored by TLC (EtOAc/Hex 10:90). Upon completion, the reaction mixture was diluted with diethyl ether and washed with saturated aqueous solution of $NH_4Cl$ followed by brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOac/Hex 5:95) to afford compound **7b** (215 mg, 56%) as yellow oil.

$^1$H NMR (200MHz, $CDCl_3$): δ = 7.85 (2H, d, *J* = 8Hz), 7.44 (2H, d, *J* = 8Hz), 7.17 (2H, s), 4.88 (2H, s), 4.69 (4H, s), 2.34 (3H, s), 1.36 (12H, s), 0.93 (18H, s), 0.08 (12H, s). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 150.98, 140.66, 134.93, 133.66, 127.85, 126.89, 83.76, 76.07, 60.26, 25.90, 24.74, 21.09, 18.33, -5.3. MS (APPI+): *m/z* calc. for $C_{34}H_{57}BO_5Si_2$: 612.40; found: 635.4 $[M+Na]^+$.

Compound **7c**

Compound **7b** (185 mg, 0.30 mmol) was dissolved in 2ml MeOH, and catalytic amount of *p*-TsOH was added. The reaction mixture was stirred for 30 min in RT, and monitored by TLC (EtOAc/Hex 80:20). Upon completion, the reaction mixture was diluted with EtOAc and washed with saturated aqueous solution of $NaHCO_3$. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 80:20) to afford compound **7c** (101 mg, 88%) as a white solid.

$^1$H NMR (200MHz, $CDCl_3$): δ =7.82 (2H, d, *J* = 8Hz), 7.40 (2H, d, *J* = 6Hz), 7.12 (2H, s), 4.87 (2H, s), 4.60 (4H, s), 2.28 (3H, s), 1.34 (12H, s). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 153.42, 141.01, 136.17, 135.40, 134.90, 130.52, 128.22, 84.97, 78.77,

61.73, 30.74, 25.89, 21.89. MS (APPI+): *m/z* calc. for $C_{22}H_{29}BO_5$: 384.2; found: 407.2 $[M+Na]^+$.

Compound **7d**

Compound **7c** (100 mg, 0.26 mmol) was dissolved in 2 ml of dry THF. Then $Et_3N$ (72 μL, 0.52mmol) and catalytic amount of DMAP was added, and the mixture was cooled to 0°C. Then, *p*-nitrophenyl-chloroformate (136 mg, 0.68 mmol) dissolved in dry THF (2 ml) was added dropwise , and the reaction was stirred for 1h at room temperature and monitored by TLC (EtOAc/Hex 25:75). Upon completion, the reaction mixture was diluted with EtOAc and washed with saturated aqueous solutions of $NH_4Cl$. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 20:80) to afford compound **7d** (111 mg, 60%) as a white solid.

$^1$H NMR (200MHz, $CDCl_3$): δ = 8.27 (4H, d, *J* = 7Hz), 7.86 ( 2H, d, *J* = 6.8Hz), 7.49 (2H,d, *J* = 6.8Hz), 7.34 (4H, d, *J* = 7Hz), 7.27 (2H, m), 5.33( 4H,s), 5.04 (2H, s), 2.38 (3H, s), 1.36 (12H ,s). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 163.23, 156.49, 153.49, 146.43, 140.78, 136.20, 135.91, 133.86, 129.15, 127.83, 127.25, 122.85, 85.22, 67.36, 31.24, 25.88, 21.81. MS (ES+): *m/z* calc. for 714.6; found: 737.6 $[M+Na]^+$.

Compound **5**

Compound **7d (**20 mg, 0.03 mmol) was dissolved in 1ml DMF and cooled to 0°C. Then $Et_3N$ (5 μL, 0.06 mmol) was added, followed by the addition of compound **7f** (17 mg, 0.03 mmol) dissolved in 1 ml DMF. The reaction was stirred at room temperature for 2h, and monitored by TLC (DCM/MeOH 90:10). After the formation of compound **7e** was complete, compound **5a** (20 mg, 0.03mmol) dissolved in 1 ml DMF was added at room temperature. The reaction mixture was stirred for additional 0.5h, and monitored by TLC (DCM/MeOH 80:20) and by RP-HPLC (10-90% ACN in $H_20$, 20 minutes). Upon completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (DCM/MeOH 90:10) to afford compound **5** (15 mg, 30%) as a blue solid.

$^1$H NMR (200MHz, $CDCl_3$): δ = 7.89 (4H, m), 7.36-7.33 (10H, m), 7.24-7.20 (2H, m), 7.12-7.05 (5H, m), 6.92-6.88 (2H. m), 6.75 (2H, m), 6.54-6.37 (3H, m), 6.26-6.16

(2H, m), 5.93-5.88 (1H, m), 5.04 (4H, s), 4.92 (2H, s), 4.10-4.07 (4H, m), 3.96-3.90 (2H, m), 3.78-3.70 (2H, m), 3.49-3.40 (4H, q, $J$ = 8Hz), 3.32 (8H, m), 2.61 (3H, s), 2.21 (4H, m), 1.67 (24H, m), 1.39-1.31 (12H, m), 1.24-1.19 (24H, m). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 182.61, 172.36, 157.65, 153.20, 151.15, 147.96, 146.8, 144.17, 143.28, 141.99, 141.06, 139.35, 134.06, 131.26, 129.85, 128.46, 127.22, 126.13, 126.51, 124.84, 122.09, 120.19, 116.87, 112.76, 110.06, 107.32, 108.67, 104.52, 104.05, 101.40, 86.24, 65.84, 62.29, 56.38, 53.61, 48.27, 45.72, 40.80, 39.77, 38.97, 35.77, 32.00, 27.89, 22.63, 15.31, 13.94, 12.11. MS (ESI+): *m/z* calc. for $C_{100}H_{130}BN_9O_9^{2+}$: 806.5; found: 806.5 $[M]^+$.

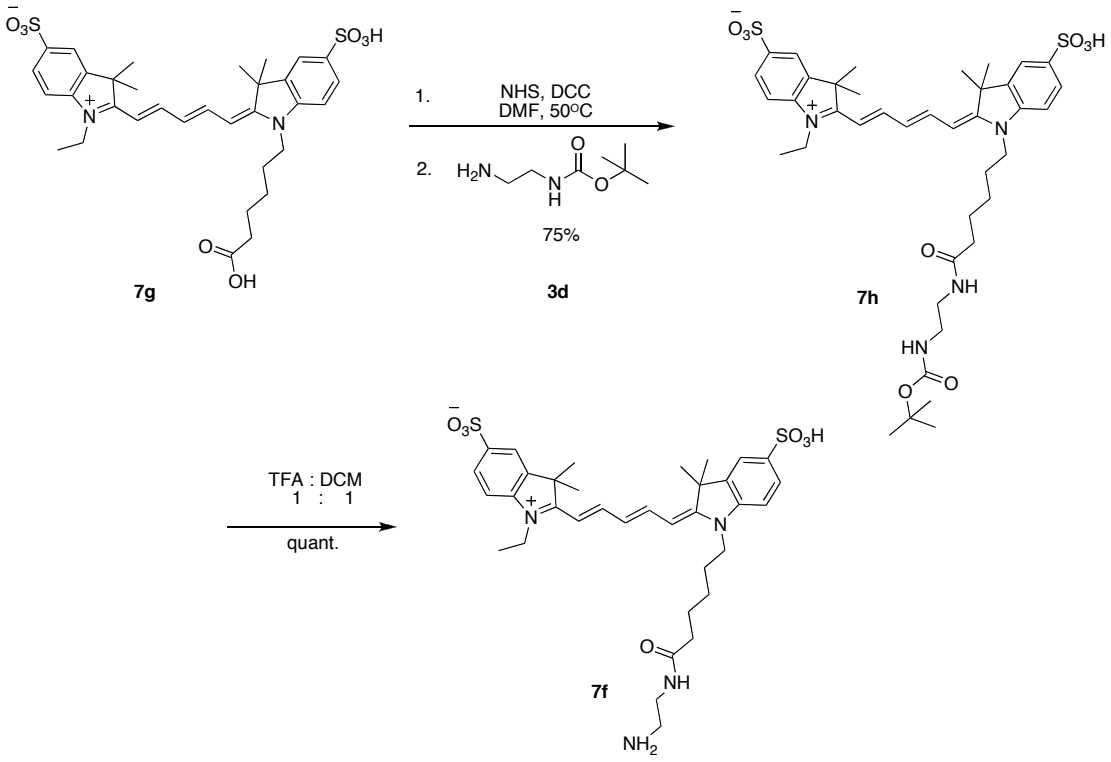


***Figure 3***: Chemical synthesis of compound **7f**.

Compound **7h**

Compound **7g**[9] (500 mg, 0.94 mmol) was dissolved in 10 ml DCM. Then DCC (291 mg, 1.41 mmol) was added followed by the addition of NHS (162 mg, 1.41 mmol). The mixture was heated to 50°C and stirred for 2h. Then compound **3d**[3] (225 mg, 1.41 mmol) was added and the mixture was stirred ON at room temperature. The reaction was monitored by TLC (DCM/MeOH 90:10). Upon completion the solvent was removed under reduced pressure and the crude product was purified by column

chromatography on silica gel (DCM/MeOH 90:10) to afford compound **7h** (728 mg, 75%) as a blue solid.

$^{1}$H NMR (200MHz, DMSO): δ = 8.43 (2H, t, *J* = 14Hz), 7.64 (2H, d, *J* = 6.6Hz), 7.39 (4H, m), 7.26-7.22 (2H, m), 6.64 (1H, t, *J* = 13Hz), 6.33 (2H, d, *J* = 14Hz), 4.15-4.09 (4H, m), 3.03-2.91 (4H, m), 2.08 (2H, t, *J* = 7.8Hz), 1.67 (12H, s), 1.57-1.50 (6H, m), 1.35 (9H, s), 1.30 (3H, t, *J* = 7Hz). $^{13}$C NMR (200MHz, DMSO): δ = 173.16, 172.52, 156.05, 154.53, 142.44, 142.01, 141.54, 128.82, 126.31, 125.06, 122.86, 111.45, 111.24, 103.54, 103.18, 77.98, 55.27, 49.25, 44.36, 35.47, 28.55, 27.41, 27.16, 26.07, 25.17, 12.49. MS (ESI+): *m/z* calc. for $C_{40}H_{55}N_4O_3^+$: 639.8; found: 639.4 $[M]^+$.

Compound **7f**

Compound **7h** (20 mg, 0.03 mmol) was treated with a mixture of TFA:DCM (1:1) for 5 minutes, which was then evaporated to afford compound **7f** in quantative yield**.** The amine was used immediately in the next step without further purification.

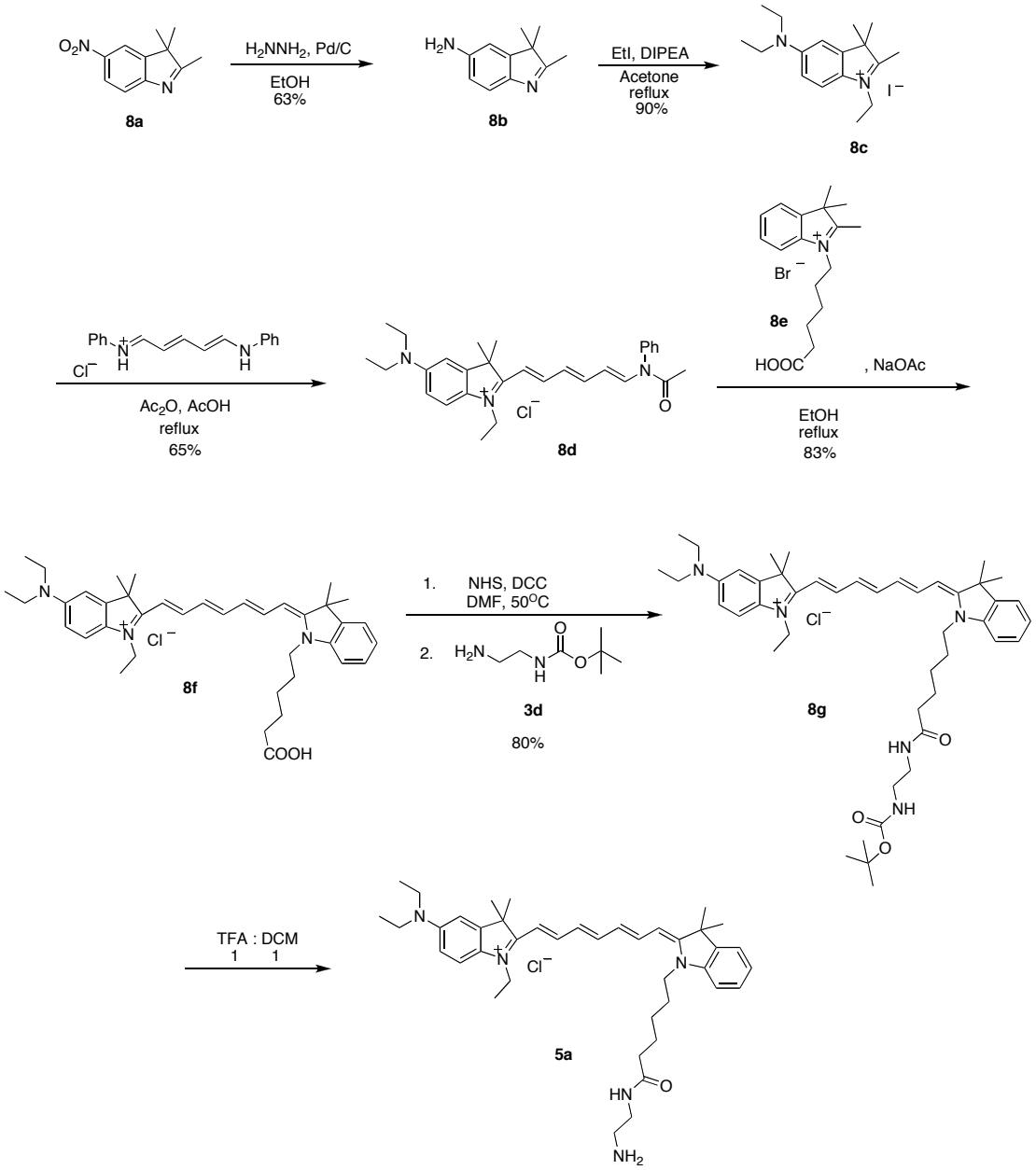


***Figure 4***: Chemical synthesis of compound **5a**.

Compound **8b**

Commercially available compound **8a** (1.4 gr, 6.86 mmol ) was dissolved in 10 ml EtOH. Then 1gr of an aqueous solution of $H_2NNH_2$ (35%) was added dropwise. The mixture was stirred for 10 minutes, followed by the slow addition of Pd/C (390 mg). The mixture was heated to 70°C and stirred for 1h. The reaction was monitored by TLC (EtOAc/Hex 50:50). Upon completion, the mixture was filtered with a sinter glass, and the solvent was removed under reduced pressure. The crude product was

purified by column chromatography on silica gel (EtOAc/Hex 70:30) to afford compound **8b** (750 mg, 63%) as a yellow solid.

$^{1}$H NMR (200MHz, $CDCl_3$): δ = 7.27 (2H, d, *J* = 8Hz), 6.65 (2H, m), 4.07 (2H, brs), 2.2 (3H, s), 1.25 (6H, s). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 184.57, 146.84, 144.83, 144.44, 119.56, 113.89, 108.95, 53.15, 22.90, 14.57. MS (ESI+): *m/z* calc. for $C_{10}H_{12}N_2$:160.1; found: 161.1 $[M+H]^+$.

Compound **8c**

Compound **8b** (750 mg, 4.31 mmol) was dissolved in 7 ml Acetone. Then DIPEA (1.65 ml, 9.48 mmol) was added, followed by the addition of ethyl iodide (1.72 ml, 21.5 mmol). The reaction mixture was heated to reflux and stirred ON. Upon completion, the solvent was removed under reduced pressure. Then the crude product was diluted with DCM and washed with $H_2O$. The organic layer was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (DCM/MeOH 90:10) to afford compound **8c** (1.5 gr, 90%) as a greed solid.

$^{1}$H NMR (200MHz, $CDCl_3$): δ =7.53 (1H, d, *J* = 10Hz), 7.18 (1H, m), 6.72 (1H, m), 4.66 (2H, q, *J* = 8Hz), 3.49 (4H, q, *J* = 8Hz), 2.94 (3H, s), 1.59 (6H, s), 1.24 (9H, m). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 185.97, 149.21, 143.91, 128.8, 115.95, 110.91, 104.93, 44.81, 44.52, 23.33, 15.27, 14.59, 12.12, 8.22. MS (ESI+): *m/z* calc. for $C_{16}H_{25}N_2^+$: 245.2; found: 245.2 $[M]^+$.

Compound **8d**

Compound **8c** (1.5 gr, 3.88 mmol) was dissolved in 5 ml of AcOH and 5 ml of $Ac_2O$. Then commercially available Glutaconaldehydedianil Hydrochloride (1.1 gr, 3.88 mmol) was added. The reaction mixture was heated to reflux, and stirred for 1h. The reaction was monitored by TLC (DCM/MeOH 90:10). Upon completion, the solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel (DCM/MeOH 90:10) to afford compound **8d** (1.5 gr , 65%) as a blue solid.

$^{1}$H NMR (200MHz, $CDCl_3$): δ = 8.05 (1H, d, *J* = 13.8Hz), 7.60- 7.36 (5H, m), 7.32-7.01 (5H, m), 6.69-6.56 (2H, m), 5.34-5.21 (1H, m), 4.55 (2H, q, *J* = 7.4Hz), 3.45 (4H, q, *J* = 7Hz), 2.24 (3H, s), 1.61 ( 6H, s), 1.45 (3H, t, *J* = 7.4Hz), 1.24 (6H, t, *J* =

7Hz). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 172.50, 169.51, 150.03, 148.95, 147.31, 145.76, 138.96, 130.49, 129.72, 128.42, 128.12, 123.47, 119.94, 115.12, 113.35, 112.42, 111.6, 104.48, 50.99, 44.93, 42.25, 27.14, 26.84, 13.85, 12.26. MS (ESI+): *m/z* calc. for $C_{30}H_{38}N_3O^+$: 456.3; found: 456.3 $[M]^+$.

Compound **8f**

Compound **8d** (640 mg, 1.09 mmol) was dissolved in 20 ml EtOH. Compound **8e**[9] (389 mg ,1.09 mmol) was then added, followed by the addition of NaOAc (181 mg, 2 mmol). The mixture was heated to reflux and stirred for 0.5h. The reaction was monitored by TLC (DCM/MeOH 90:10). Upon completion the solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (DCM/MeOH 90:10) to afford compound **8f** (606 mg , 83%) as a blue solid.

$^1$H NMR (200MHz, $CDCl_3$): δ = 7.80 (4H, d, *J* = 8Hz), 7.29-7.18 (5H, m), 7.00-6.96 (3H, m). 6.87 (1H, d, *J* = 8Hz), 5.78 (1H, d, *J* = 7.4Hz), 4.12 (2H, m), 3.76 (2H, m), 3.53-3.41 (4H, m), 2.41 (2H, t, *J* = 6.6Hz), 1.70 (2H, m), 1.66 (6H, s), 1.60 (6H, s), 1.44 (4H, m), 1.21-1.14 (9H, m). $^{13}$C NMR (200MHz, $CDCl_3$): δ = 176.62, 170.01, 144.24, 142.82, 139.70, 138.60, 130.22, 139.70, 138.37, 123.52, 122.62, 121.81, 120.09, 113.03, 111.60, 108.46, 105.41, 65.67, 50.08, 47.48, 45.04, 33.86, 28.03, 27.55, 26.24, 24.40, 13.02, 12.26. MS (ESI+): *m/z* calc. for $C_{39}H_{52}N_3O_2^+$: 594.4; found: 594.4 $[M]^+$.

Compound **8g**

Compound **8f** (640 mg, 1.07 mmol) was dissolved in 10 ml DMF. Then DCC (333 mg, 1.61 mmol) was added followed by the addition of NHS (185 mg, 1.61 mmol). The mixture was heated to 50°C and stirred for 2h. Then compound **3d**[3] (258 mg, 1.61 mmol) was added and the mixture was stirred ON at room temperature. The reaction was monitored by TLC (DCM/MeOH 90:10). Upon completion the solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (DCM/MeOH 90:10) to afford compound **8g** (660 mg, 80%) as a blue solid.

$^1$H NMR (200MHz, $CD_3OD$): δ = 8.06 (1H, m), 7.63-7.43 ( 1H, m) , 7.31-7.25 ( 4H, m), 7.04-6.96 (3H, m), 6.87 (2H, brs), 6.57 (2H, m), 5.82 (1H, m), 4.26 (2H, m), 3.85

(2H, m), 3.47-3.40 (4H, m), 3.22 (2H, d, $J$ = 4.8Hz), 3.15 (2H, d, $J$ = 4.8Hz), 2.21 (2H, t, $J$ = 8Hz), 1.68 (6H, s), 1.59 (6H, s), 1.42 (9H, m), 1.33-1.26 (6H, m), 1.20 (9H, t, $J$ = 6.8Hz). $^{13}$C NMR (200MHz, $CD_3OD$): δ = 174.71, 160.04, 158.32, 157.03, 147.78, 143.23, 139.69, 133.25, 130.12, 127.84, 121.95, 121.53, 113.18, 111.34, 108.24, 105.33, 99.72, 78.60, 50.14, 44.42, 42.29, 39.45, 39.02, 35.39, 33.26, 29.27, 27.33, 27.08, 26.24, 26.01, 25.26, 24.58, 11.39. MS (ESI+): *m/z* calc. for $C_{46}H_{66}N_5O_3^+$: 737.5; found: 737.5 $[M]^+$.

Compound **5a**

Compound **8h** (22mg, 0.03 mmol) was treated with a mixture of TFA:DCM (1:1) for 5 minutes, which was then evaporated to afford compound **5a** in quantative yield**.** The amine was immediately used in the next step without further purification.

Fluorescence spectra of probes **2**, **4** and **5**

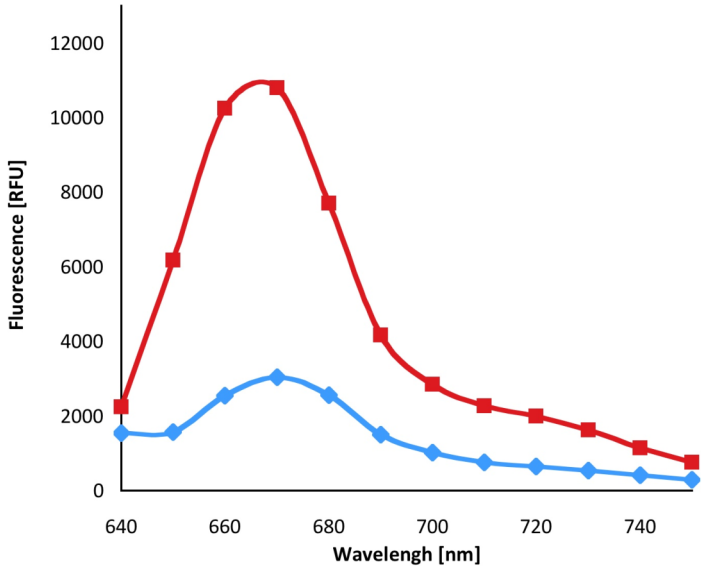


***Figure 5***: Fluorescence spectrum (λex=640 nm) of probe **2** (blue) [10 μM], **Cy5** (red), [20 μM] in PBS buffer pH=7.4.

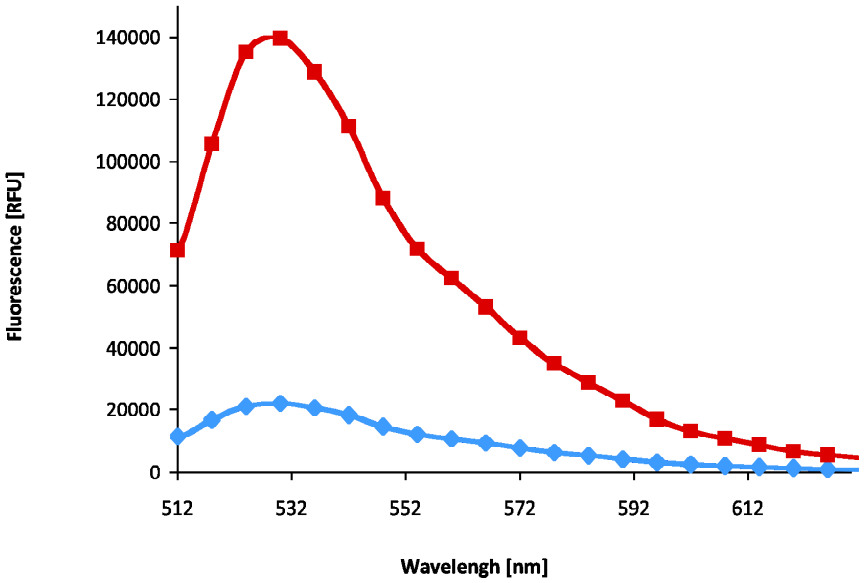


***Figure 6***: Fluorescence spectrum (λex=490 nm) of probe **4** (blue), mixture (1:1) of compound **4a** and **DR1** (red), [40 µM] in 12% DMSO in PBS buffer pH=7.2.

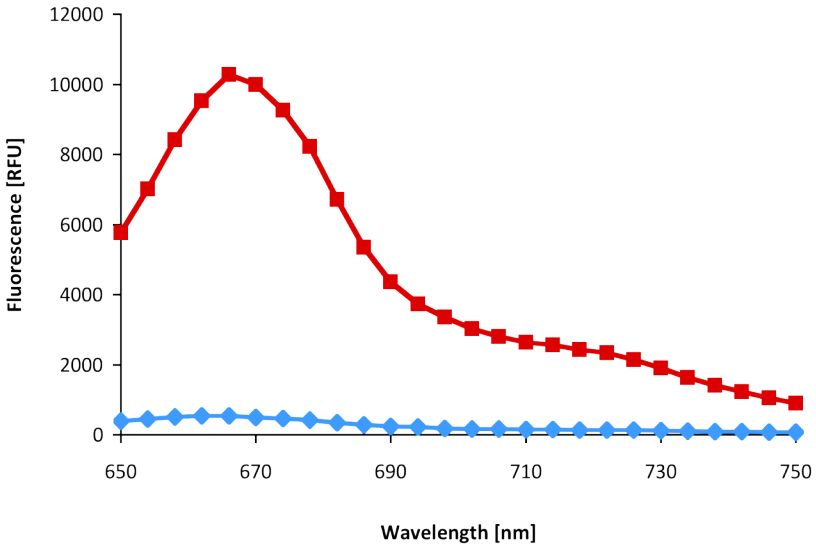


***Figure 7***: Fluorescence spectrum (λex=600 nm) of probe **5** (blue), **Cy5** (red), [30µM] 20% DMSO in PBS buffer pH=8.3.

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