Supporting Information

Turn-on Fluorescence Switch Involving Aggregation and Elimination Processes for β-Lactamase-Tag

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CONTENTS:

1. Materials and Instruments

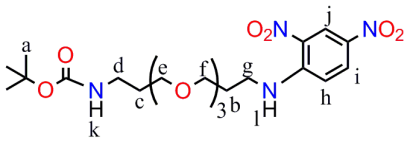
General Chemicals and biological reagent were similar to our previous report.[S1] 7-Amino-3-chloromethyl-3-cephem-4-carboxylic acid *p*-methoxybenzyl ester hydrochloride (ACLE·HCl) was obtained from Otsuka Chemical Co. Ltd.

NMR spectra were recorded on a JEOL JNM-AL400 instrument at 400 MHz for ^{1}H and at 100.4 MHz for ^{13}C NMR, using tetramethylsilane as an internal standard. Mass spectra were measured on a JEOL JMS-700 for FAB. UV-Visible absorbance spectra were measured using a Shimadzu UV1650PC spectrometer. Fluorescence spectra were measured using a Hitachi F4500 spectrometer. Slit width was 2.5 nm for both excitation and emission, and the photomultiplier voltage was 700 V. Fluorescence microscopic images were recorded using an IX71 inverted fluorescence microscope (Olympus), a Cool Snap HQ cooled CCD camera (Roper Scientific), and a USH-103OL mercury lamp (Olympus). The filter sets used were Olympus BP330-385, DM455, and BA455 for Hoechst 33342; BP470-495, DM505, and BA510-550 for fluorescein derivatives. MetaMorph imaging software (Universal Imaging Corporation) was used for imaging and data analysis. Silica gel column chromatography was performed using BW-300 (Fuji Silysia Chemical Ltd.). Fluorescence images of SDS-PAGE were visualized using an AE-6935B VISIRAYS-B (ATTO).

2. Syntheses of Compounds (Scheme S1)

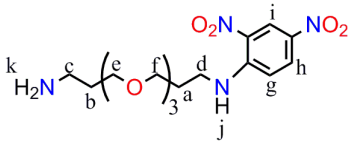
Synthesis of 1. Compound **1** was synthesized according to the procedure available in literature.[S2]

Synthesis of 2. 2,4-Dinitrofluorobenzene (6.36 g, 34 mmol) in dichloromethane (5 mL) was added to the dichloromethane solution (5 mL) of **1** (7.3g, 23 mmol). The mixture was then set for reflux at 50 ˚C. After 3 h of the desired product was isolated by flash column chromatography using 50% ethyl acetate in hexane. Compound **2** was isolated as yellow liquid after complete removal of solvent (7.7 g, y. 73%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 9H, 9 × a), 1.76 (t, 2H, 2 × b), 2.05 (t, 2H, 2 × c), 3.22 (m, 2H, 2 × d), 3.52-3.72 (m, 14H, 4 × e, 2 × g, 8 × f), 4.92 (br, 1H, k), 6.97 (d, 1H, h), 8.26 (m, 1H, i), 8.91 (br, 1H, k), 9.14 (s, 1H, j); ^{13}C NMR (DMSO-d_6 100 MHz) δ 28.4 (a), 28.7, 29.7 (b, c), 38.5, 41.9 (d, g), 69.2, 69.5, 70.2, 70.5 , 70.6, 70.7 (e, f), 78.9 ($(CH_3)C$-O), 113.8, 124.3, 130.3, 135.8, 148.4 (Ar), 156.0 (C=O); HRMS ($C_{21}H_{35}N_4O_9$, FAB+); Found 487.2394 ; Calc., 487.2404.



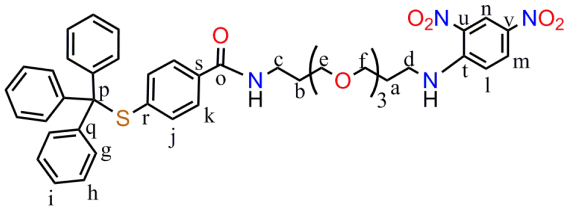
Synthesis of 3. 25 mL of trifluoroacetic acid (TFA) was added to the stirring solution of **2** (7.7 g, 16 mmol) in dichloromethane (25 mL). After 90 min the solvent was evaporated to obtain the desired product **3** (7.6 g, y. 96%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.97-2.12 (m, 4H, 2 × a, 2 × b), 3.25-3.34 (m, 2H, 2 × c), 3.52 (t, 2H, 2 × d) 3.66-3.84 (m, 12H, 4 × e, 8 × f), 6.93 (d, 1H, g), 7.13 (br, 1H, k), 8.26 (m, 1H, h), 8.97 (br, 1H, j), 9.10 (s, 1H, i); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 26.2 (b), 28.1 (a), 40.9 (c), 42.4 (d), 69.6 (e), 70.4 (f), 113.9, 124.2,

130.2, 130.4, 135.8, 148.4 (Ar); HRMS ($C_{16}H_{27}N_4O_7$, FAB+); Found 387.1880 ; Calc., 387.1874

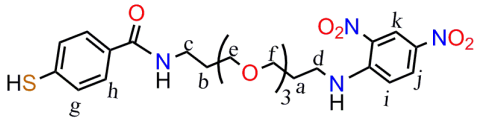


Synthesis of I. Compound **I** was synthesized according to the procedure available in literature.[S3]

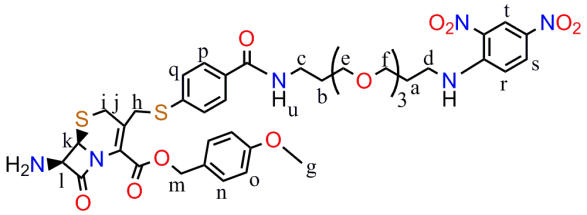
Synthesis of 4. Triethylamine (TEA) (30 mL) was added to the stirring solution of **I** (7.9 g, 20 mmol) in dichloromethane (25 mL) at 0˚C. To this 1-hydroxy-benzotriazole (HOBt, 2.7 g, 20 mmol) and water soluble carbodiimide (WSCD, 3.8 g, 20 mmol) were added subsequently. Compound **3** (**3**.TFA, 5 g, 10 mmol) was added and the stirring was continued at 0˚C. After 12 h the reaction was set up at RT for another 12 h. The desired product was isolated by flash column chromatography using 80% ethyl acetate in hexane. Compound **4** was isolated as yellow liquid after complete removal of solvent (3.6 g, y. 47%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.83 (t, 2H, 2 × a), 1.96 (t, 2H, 2 × b), 3.47-3.55 (m, 8H, 2 × c, 2 × d, 4 × e), 3.59-3.63 (m, 8H, 8 × f), 6.90 (d, 1H, l), 6.96 (d, 2H, 2 × k), 6.98 (br, 1H, NH), 7.20-7.27 (m, 9H, 6 × h + 3 × i), 7.35-7.37 (m, 6H, 6 × g), 7.42 (d, 2H, 2 × j), 8.21 (d, 1H, m), 8.85 (br, 1H, NH), 9.12 (d, 1H, n); ^{13}C NMR (DMSO-d_6 100 MHz) δ 28.5 (a), 28.7 (b), 39.0 (c), 42.0 (d), 69.2 (p), 70.1, 70.3 (e), 70.8 (f), 113.8 (l), 124.3, 126.6, 127.0, 127.8, 129.9, 130.3, 132.2, 132.9, 135.5, 139.3 (g, h, i, j, k, m, n, s, u, v), 144.0 (q), 148.3 (t), 166.4 (o); HRMS ($C_{42}H_{45}N_4O_8S$, FAB+); Found 765.2950; Calc., 765.2958.



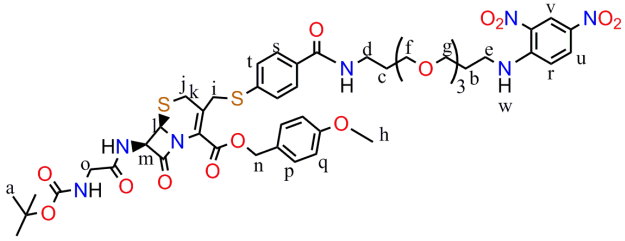
Synthesis of 5. Compound **4** (3.5 g, 5 mmol) was dissolved in a mixture of 50% TFA in dichloromethane (total volume 50 mL) and triisopropylsilane (25 mL). After stirring the mixture for 3 h, solvent was removed under reduced pressure. The yellow mass was washed vigorously with *n*-hexane and dried under vacuum to obtain **5** (2 g, y. 84%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.87 (t, 2H, 2 × a), 1.98 (t, 2H, 2 × b), 3.46-3.62 (m, 16H, 2 × c, 2 × d, 4 ×e, 8 × f), 6.90 (d, 1H, i), 7.28 (d, 2H, 2 × k), 7.64 (d, 2H, 2 × j), 8.24 (d, 1H, j), 8.85 (br, 1H, NH), 9.12 (s, 1H, k); ^{13}C NMR (DMSO-d_6 100 MHz) δ 28.3 (a), 29.3 (b), 36.8 (c), 41.1 (d), 68.3 (e), 69.7 (f), 115.2, 123.6, 126.3, 127.6, 128.2, 129.1, 130.1, 133.6, 134.6, 138.7, 148.3, 158.2 (Ar), 165.3 (C=O); HRMS ($C_{23}H_{31}N_4O_8S$, FAB+); Found 523.1862; Calc., 523.1857.



Synthesis of 6. ACLE hydrochloride (1.3 g, 3 mmol) was suspended in 90 mL dichloromethane at 0 ˚C. TEA (0.6 g, 6 mmol) was slowly added in three portions over the period of 20 min to the suspension. N-methylmorpholine (NMM, 0.4 g, 4 mmol) and compound **5** (1.5 g, 3 mmol) were added sequentially. The resulting mixture was stirred at 0 ˚C for 2 h. The desired product **6** was obtained by flash column chromatography with 2% methanol in dichloromethane (1.3 g, y. 53%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.87 (t, 2H, 2 × a), 1.98 (t, 2H, 2 × b), 3.47-3.82 (m, 23H, 2 × c, 2 × d, 4 ×e, 8 × f, 3 × g, 2 × h, i, j), 5.15 (m, 3H, k, 2 × m), 5.31 (s, 1H, l), 6.87 (m, 2H, 2 × n), 6.93 (d, 1H, r), 7.30 (m, 4H, 2 × o, 2 × q), 7.68 (m, 2H, 2 × p), 8.24 (d, 1H, s), 8.85 (br, 1H, u), 9.11 (s, 1H, t); ^{13}C NMR (DMSO-d_6 100 MHz) δ 28.3 (a), 29.4 (b), 35.8 (h), 36.7 (c), 41.0 (d), 52.4 (g), 53.4 (l), 62.5 (k) 63.3 (m), 68.3 (e), 69.7 (f), 123.8, 127.8 (C=C), 113.4, 113.9, 115.2, 128.1, 129.6, 130.1, 130.5,134.4, 148.4, 158.2, 159.4 (Ar), 162.3 (t), 166.4, 175.7 (C=O); HRMS ($C_{39}H_{47}N_6O_{12}S_2$, FAB+); Found 855.2683; Calc., 855.2688.

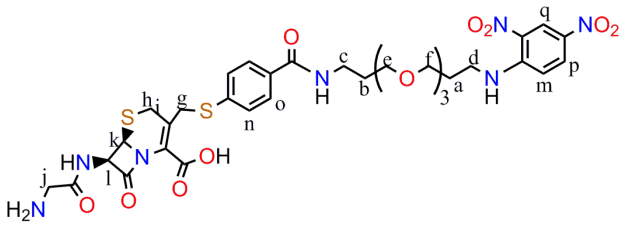


Synthesis of 7. TEA (177 mg, 1.8 mmol) was added to the dichloromethane solution (30 mL) of Boc-protected glycine (307 mg, 1.8 mmol) at 0 ˚C. To this reaction mixture HOBt (237 mg, 1.8 mmol) and WSCD (336 mg, 1.8 mmol) were added sequentially. The stirring was continued for 1 hr. Compound **6** (484 mg, 0.6 mmol) was added to the reaction mixture. After 3 h stirring at 0 ˚C, the reaction was continued at RT for another 21 h. The desired product **7** was isolated by flash column chromatography using 100% ethyl acetate (520 mg, y. 91%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.36 (s, 9H, 9 × a), 1.72 (t, 2H, 2 × b), 1.87 (t, 2H, 2 × c), 3.29-3.58 (m, 20H, 2 × d, 2 × e, 4 ×f, 8 × g, 2× i, j, k), 3.73 (s, 3H, 3 × h), 4.01 (br, 1H, NH), 5.04-5.17 (m, 4H, l, m, 2× n), 7.18 (d, 1H, r), 7.31 (m, 2H, 2 × p), 7.38 (m, 2H, 2 × q, 2 × t), 7.73 (m, 2H, 2 × s), 8.22 (m, 1H, u), 8.82 (s, 1H, v); ^{13}C NMR (DMSO-d_6 100 MHz) δ 28.2 (a), 28.3 (b), 29.4 (c), 36.7 (j, k), 41.04 (d), 41.8 (e), 42.8 (i), 49.8 (o), 52.7 (h), 55.1 (m), 59.8 (l), 67.3 (n), 68.3 (e), 69.8 (f), 79.5 ((CH$_3$)C-O), 123.6, 124.5 (C=C), 109.6, 113.7, 115.1, 119.2, 127.4, 127.8, 129.6, 130.2,131.9, 132.5, 134.6, 138.6, 139.1, 148.2, 155.8, 159.3 (Ar), 163.9, 165.5, 167.0, 170.2, 171.8 (C=O); HRMS ($C_{46}H_{58}N_7O_{15}S_2$, FAB+); Found 1012.3421; Calc., 1012.3427.

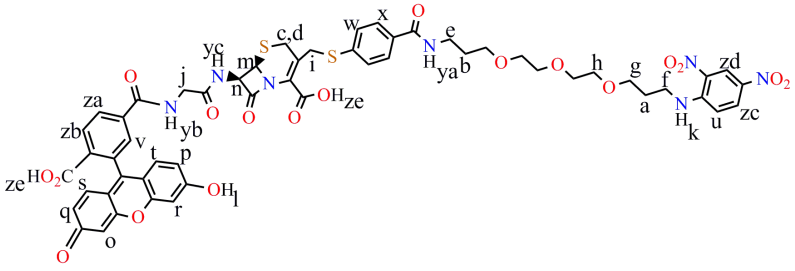


Synthesis of 8. Compound **7** (400 mg) was dissolved in 30 mL of dichloromethane. To this 8 mL of thioanisole was added at 0 ˚C. To this stirring mixture 24 mL of TFA was added. The reaction was continued with stirring at 0 ˚C for 18 h. After evaporation of the solvent, precipitate was obtained with the addition of 150 mL diethylether. The precipitate was

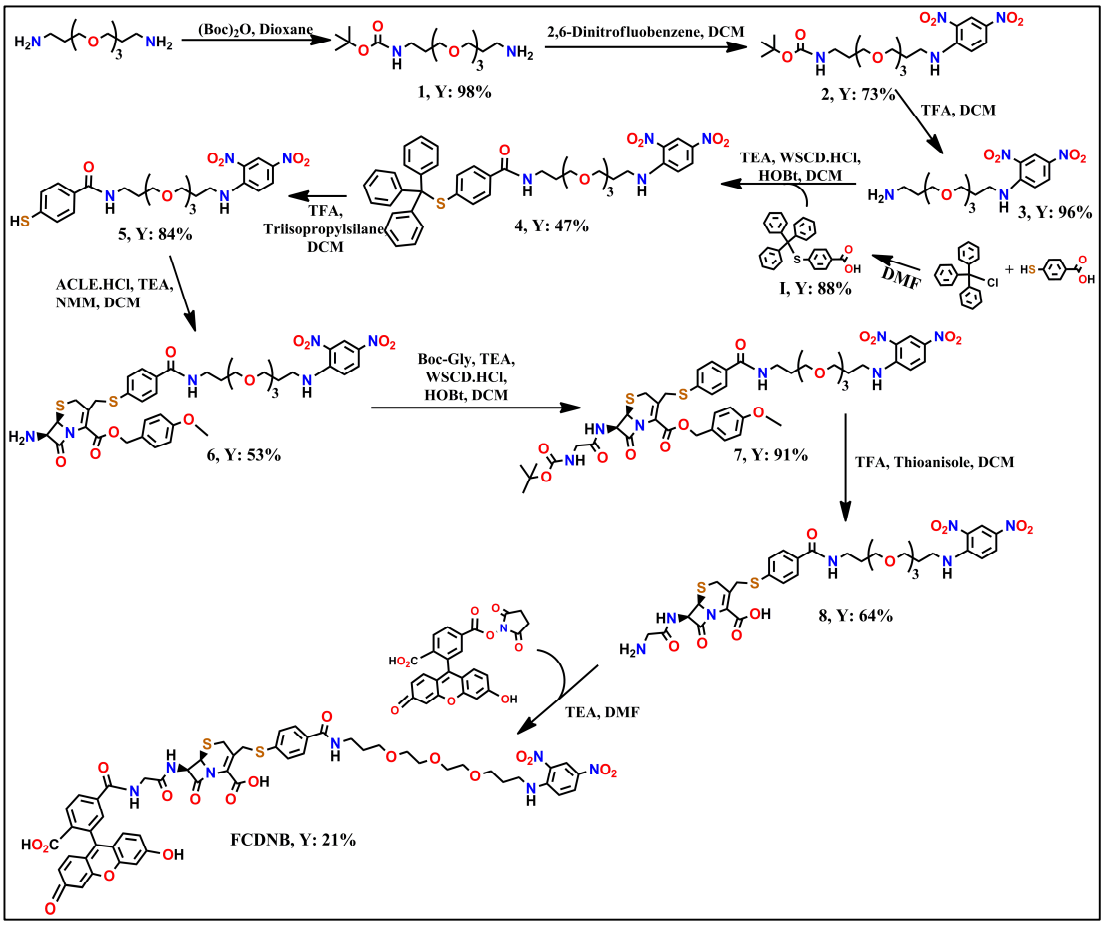
washed with 300 mL of diethylether and dried under vacuum to obtain **8** (200 mg, y. 64%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.72 (t, 2H, 2 × a), 1.86 (t, 2H, 2 × b), 3.42-3.58 (m, 22H, 2 × c, 2 × d, 4 ×e, 8 × f, 2 × g, h, i, 2 × j), 5.18 (d, 1H, k), 5.46 (d, 1H, l), 7.16 (d, 1H, m), 7.22 (d, 2H, 2 × n), 7.69 (d, 2H, 2 × o), 8.21 (d, 1H, p), 8.81 (s, 1H, q); ^{13}C NMR (DMSO-d_6 100 MHz) δ 28.2 (a), 29.4 (b), 36.7 (c), 41.0 (d), 49.9 (h), 52.4 (g), 60.1 (j), 68.3 (e), 69.6 (f), 69.7 (k), 69.8 (l), 123.6, 124.8 (C=C), 109.6, 115.1, 119.6, 125.9, 127.4, 128.2, 130.0, 132.2, 134.6, 138.1, 139.3, 148.2 (Ar), 163.2, 165.5, 166.9, 169.0 (C=O); HRMS ($C_{33}H_{42}N_7O_{12}S_2$, FAB+); Found 792.2317; Calc., 792.2327.



Synthesis of FCDNB. Compound **8** (32 mg, 0.4 mmol) and 6-carboxyfluorescein-succinimidyl ester (19 mg, 0.4 mmol) were dissolved in 0.5 mL of *N*,*N*-dimethylformamide. To this solution TEA (4 mg, 0.4 mmol) was added at 0 ˚C. The reaction was continued for 6 h. The desired product **FCDNB** was isolated using preparative HPLC (10 mg, y. 21%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.72 (m, 2H, 2 × a), 1.87 (m, 2H, 2 × b), 3.48-3.56 (m, 18H, c, d, 2 × e, 2 × f, 4 × g, 8 × h), 3.80-3.89 (m, 4H, 2 × i, 2 × j), 4.07 (d, 1H, k), 5.04 (s, 1H, l), 5.12 (d, 1H, m), 5.42 (m, 1H, n), 6.54-6.61 (m, 4H, o, p, q, r), 6.69 (d, 2H, s, t), 7.21 (d, 1H, u), 7.37 (d, 2H, 2 × w), 7.68 (s, 1H, v), 7.74 (d, 2H, 2 × x), 8.08 (d, 1H, za), 8.16 (d, 1H, zb), 8.24 (dd, 1H, zc), 8.39 (m, 1H, ya), 8.85 (d, 1H, zd), 8.93-9.01 (br, 2H, yb, yc), 10.14 (br s, 2H, ze); ^{13}C NMR (DMSO-d_6 100 MHz) δ 21.2 (i) 27.9 (a, b), 41.0 (c), 41.4 (d), 45.6 (j), 66.9 (e), 68.4 (f), 69.9 (g), 78.9 (h), 98.7 (m), 102.3 (n), 123.7, 127.6 (C=C), 109.0, 111.1, 115.0, 116.6, 128.1, 128.8, 130.0, 132.5, 133.4,134.6, 139.8, 140.7, 143.7, 148.3,152.0, 159.6 (Ar), 165.0, 165.4, 168.0, 169.3, 172.7, 180.1 (C=O); HRMS ($C_{54}H_{52}N_7O_{18}S_2$, FAB+); Found 1150.2828; Calc., 1150.2805.



Synthesis of FA. Synthesis of **FA** was described elsewhere.[S4]



Scheme S1. Synthetic route to **FCDNB**.

3. Preparation of Proteins

All procedures were similar to our earlier experiments.[S1]

4. Labeling Experimental Procedures

HPLC Analysis. Preparative HPLC was performed with an Inertsil ODS-3 (10.0 mm × 250 mm) column (GL Sciences Inc.) using an HPLC system that comprised a pump (PU-2087, JASCO) and a detector (UV-2075, JASCO).

Detection of protein labeling by SDS-PAGE. WT TEM-1 or BL (10 μM) was added to a solution of FCDNB (20 μM) in 100 mM HEPES buffer (pH 7.4) at 25 °C. After 30 min, labeled protein was solubilized in 2 × SDS gel loading buffer (100 mM Tris-HCl buffer (pH 6.8), 2.5% SDS, 20% glycerol, and 10% mercaptoethanol). Fluorescence images of the gels were then captured using a digital camera (Nikon COOLPIX P6000). The gels were stained with Coomassie Brilliant Blue (CBB), and images of the stained gels were captured (Figure 1a). Purified BL (10 μM) was mixed with HEK293T cell lysate, which was prepared by

freeze thaw method. The mixture was added to a solution of FCDNB (20 μM) in 100 mM HEPES buffer (pH 7.4) at 25 °C. After 30 min, labeled protein was analyzed (Figure 1b).

Fluorescence quantum yield. The fluorescent probe was dissolved in DMSO to obtain 10 mM stock solution; this solution was then diluted to the desired final concentrations by using buffer solutions. The fluorescence quantum yield of the probe was estimated in 100 mM HEPES buffer (pH 7.4), 100 mM Tris buffer (pH 7.4), PBS (pH 7.4) using a fluorescence standard, fluorescein in 0.1 M NaOH aq. (ϕ = 0.85)[S5] as a reference for comparison. The different pH buffer solutions, for pH profile study of fluorescence quantum yield, were synthesized according to our previous method.[S6]

Fluorometric assay. Enzyme assays for the quantification of WT TEM-1 and BL were performed in 100 mM HEPES buffer (pH 7.4) at RT. A total of 1.5 μL purified enzyme (20 μM) was added to 300 μL of buffer containing probes (0.5 μM). The samples were excited at 507 nm, and the fluorescence intensity enhancement was monitored at 520 nm (Figure 2b, S4).

Labeling of cell surface protein (BL-EGFR) with FCDNB. HEK293T cells maintained in 10% FBS in DMEM (Invitrogen) at 37 ºC under 5% CO_2 were transfected with the pcDNA3.1(+)-BL-EGFR plasmids using Lipofectamine 2000 (Invitrogen). After 5–6 h, the culture medium was replaced with DMEM (without phenol red), and the cells were incubated at 37 ºC for 24 h. Then, the cells were washed three times with DMEM and incubated with 5 μM FCDNB for 1 h in a CO_2 incubator. After the culture medium was replaced, microscopic images were acquired. MetaMorph imaging software (Universal Imaging Corporation) was used for imaging and data analysis.

5. Supporting Figures

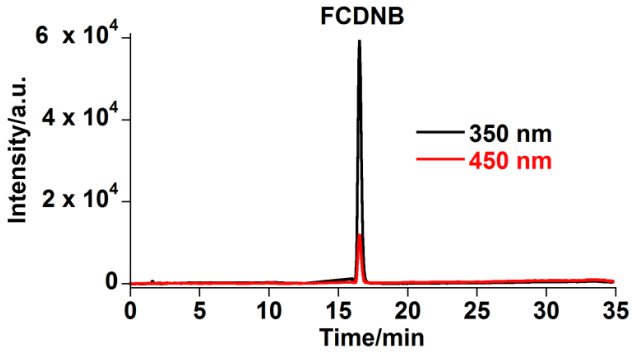


Figure S1. Analytical HPLC data of **FCDNB** for purity assay with the following eluent condition

Time (min)	Percentage of eluent A	Percentage of eluent B
0	40	60
25	30	70
30	10	90
35	40	60

eluent A: 0.1% formic acid in water
eluent B: 0.1% formic acid in acetonitrile

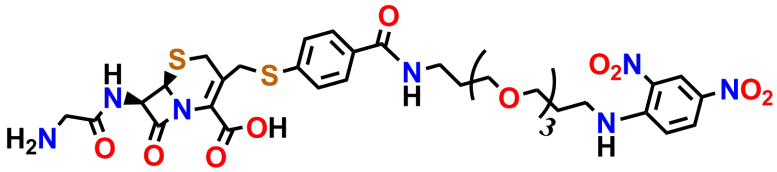


Figure S2. Molecular structure of compound **8**

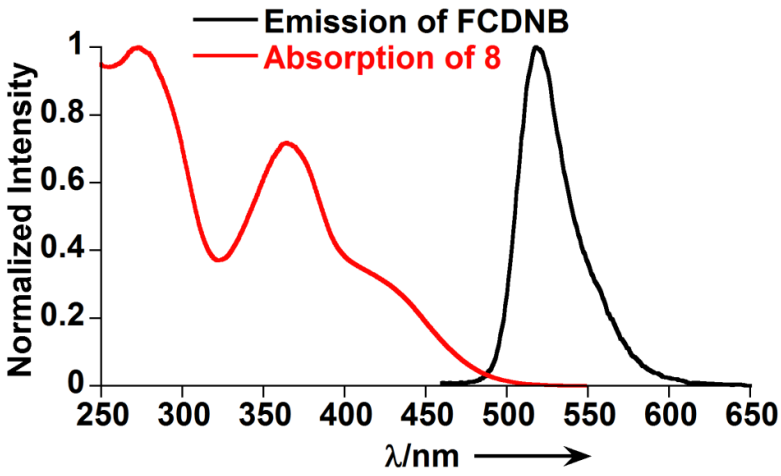


Figure S3. Normalized absorption spectra of **8** and normalized emission spectra of **FCDNB** (λ_{ex} = 440 nm)

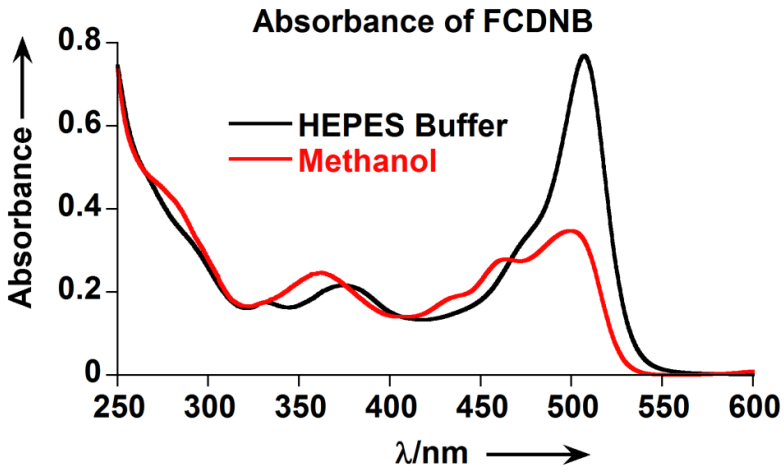


Figure S4. Absorption spectra of **FCDNB** in 100 mM HEPES buffer and methanol (Conc. of **FCDNB** = 5 μM)

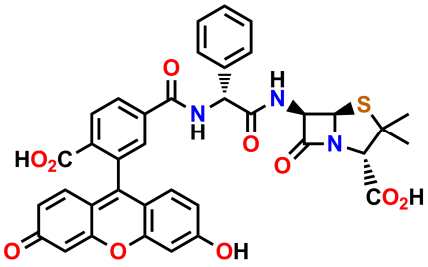


Figure S5. Molecular structure of compound **FA**

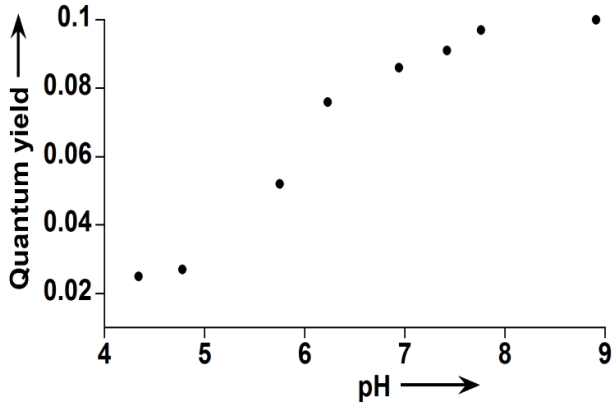


Figure S6. A pH profile of fluorescence quantum yields of **FCDNB**. The buffer solution pH were adjusted with mixing of different sodium phosphate buffer.

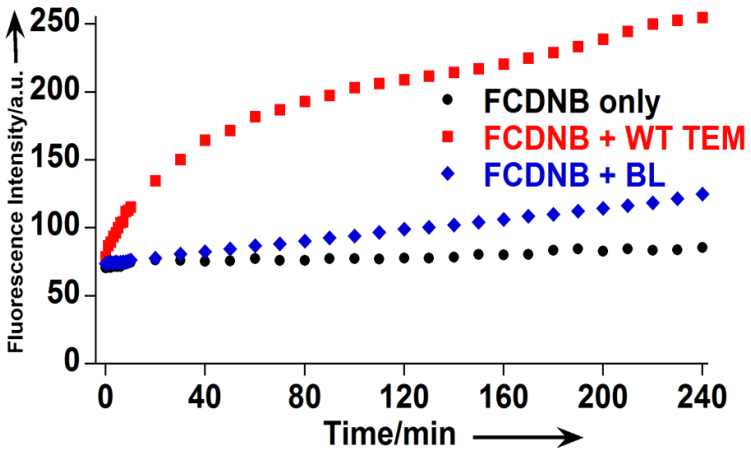


Figure S7. Change of fluorescence intensity of **FCDNB** with time

6. Supporting Table

Table S1. Fluorescence quantum yields of **FCDNB** and **FA** in different aqueous buffer solutions.

	Quantum yield of **FCDNB**	Quantum yield of **FA**
100 mM HEPES (pH 7.4)	0.05	0.71[S4]
100 mM Tris buffer (pH 7.4)	0.09	0.73
PBS (pH 7.4)	0.06	0.67

7. Supporting References

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