Supplemental Information

Bodipy-Diacrylate Imaging Probes for Targeted Proteins inside Live cells†

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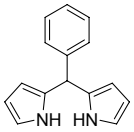
Material and Method

All reactions were performed in oven-dried glassware under a positive pressure of nitrogen. Unless otherwise noted, starting materials and solvents were purchased from Aldrich and Acros organics and used without further purification. Amino acids, Rink amide MBHA resin, and coupling reagents for preparation of peptides were purchased from peptide international, Inc. Analytical TLC was carried out on Merck 60 F254 silica gel plate (0.25 mm layer thickness) and visualization was done with UV light. Column chromatography was performed on Merck 60 silica gel (230-400 mesh). NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. Chemical shifts are reported as δ in units of parts per million (ppm) and coupling constants are reported as a *J* value in Hertz (Hz). Mass of all the compounds was determined by LC-MS of Agilent Technologies with an electrospray ionization source. High resolution mass was recorded on a Bruker MicroTOFQ-II. CD spectra of peptide were measured by Jasco J-810 spectropolarimeter. All fluorescence assays were performed with a Gemini XS fluorescence plate reader. Spectroscopic measurements were performed on a fluorometer and UV/VIS instrument, Synergy 4 of bioteck company. The slit width was 1 nm for both excitation and emission. Relative quantum efficiencies were obtained by comparing the areas under the corrected emission spectrum. The following equation was used to calculate quantum yield

$$\Phi_x = \Phi_{st}(I_x/I_{st})(A_{st}/A_x)(\eta_x^2/\eta_{st}^2)$$

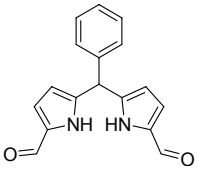
where st is the reported quantum yield of the standard, I is the integrated emission spectrum, *A* is the absorbance at the excitation wavelength, and ηis the refractive index of the solvents used. The subscript x denotes unknown and st denotes standard. 1, 3, 5, 7-tetramethyl-8-phenyl BODIPY was used as standards

1. Synthesis of Bodipy diacrylates



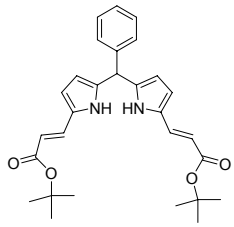
5-Phenyldipyrromethane (1a): 1a was synthesized according to the previous literature.[S1] The analytical properties are identical. ^{1}H-NMR (300 MHz. $CDCl_3$) δ 7.87 (bs, 2H, 2-NH), 7.25 (m, 5H, Ph), 6.67 (dd, J = 2.4, 4.0, 2H, 2-CH), 6.15 (dd, J = 2.8, 6.0, 2H, 2-CH), 5.90 (bs, 2H, 2-CH), 5.45 (s, 1H, -CH); ^{13}C-NMR ($CDCl_3$) δ 142.10, 132.52, 128.67, 128.43, 127.01, 117.24, 108.46, 107.26, 44.01. ESI-MS *m/z* (M+H) calc'd: 223.1, found 223.1

1,9-Diformyl-5-phenyldipyrromethane (2a): To DMF (10 mL) was added $POCl_3$ (1.5 mL, 16.4 mmol) slowly,



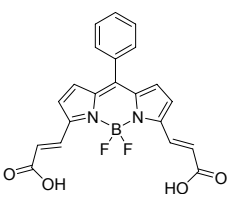
and the mixture was stirred for 5 min at 0^{o}C. This Vilsmeir reagent (7.5 mL, 10.7 mmol) was added slowly to a solution of **1a** (1.0 g, 4.5 mmol) in DMF (15 mL), and stirred for 1.5 hr at 0^{o}C. The saturated sodium acetate solution (50 mL) was added and stirred at RT overnight. After the reaction completion monitored by TLC, the reaction mixture was diluted with EtOAc, and washed with water and brine. The organic layer was dried over sodium sulfate. The filtrate was concentrated and purified by silica gel column chromatography (DCM : EtOAc = 9 : 1) to give the greenish yellow solid (1.07 g, 85%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.59 (bs, 2H, 2-NH), 9.20 (s, 2H, 2-CHO), 7.31 (m, 5H, -Ph), 6.86 (dd, *J* = 2.4, 4.0, 2H, 2-CH), 6.06 (dd, *J* = 2.4, 3.2, 2H, 2-CH), 5.59 (s, 1H, -CH); ^{13}C-NMR ($CDCl_3$) δ 179.02, 141.64, 139.16, 132.67, 129.00, 128.47, 127.72, 122.31, 111.63, 44.46; ESI-MS *m/z* (M+H) calc'd: 279.1, found 279.1

1,9-bis(*tert*-butoxycarbonylethenyl)-5 Phenyldipyrromethane (3a): To a solution of **2a** (500 mg, 1.80 mmol) in DCM (20 mL) was added (*tert*-butoxycarbonylmethylene)triphenylphosphorane (2.03 g,



5.40 mmol) at 0 ^{o}C, and the mixture was stirred at RT overnight. The reaction mixture was diluted with DCM and washed with brine. The organic layer was dried over sodium sulfate, filtered, concentrated, and purified by silica gel column chromatography (EtOAc : DCM = 1 : 40) to give a greenish yellow solid **3a** (375 mg, 44 %) ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.37 (bs, 2H, 2-CH), 7.32 (d, *J* = 16.0, 2-CH), 7.26 (m, 5H, -Ph), 6.42 (app t, *J* = 2.8, 2H, 2-CH), 5.94 (app t, *J* = 2.8, 2H, 2-CH), 5.77 (d, *J* = 16.0, 2H, 2-CH), 5.45 (s, 1H, -CH); ^{13}C-NMR ($CDCl_3$); 166.91, 140.24, 136.36, 132.95, 129.02, 128.52, 128.32, 127.63, 114.68, 113.00, 110.38, 80.12, 44.33, 28.25; ESI-MS *m/z* (M+H) calc'd: 475.2, found 475.2

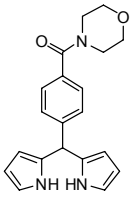
Bodipy diacrylate 4a: To a solution of **3a** (200 mg, 0.421



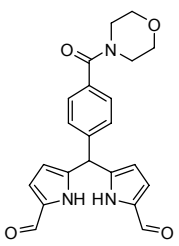
mmol) in DCM (35 mL) was added DDQ (144 mg, 0.636 mmol). After stirring for 15 min at RT, the mixture was cooled to 0 ^{o}C. To this mixture, DIEA (2 mL, 11.6 mmol) and BF_3OEt_2 (1.4 mL, 11.1 mmol) were added and slowly warmed up to RT while stirring for 2 hrs. The reaction mixture was diluted with DCM, and washed with aq. $NaHCO_3$ and brine. The organic layer was dried over sodium sulfate and the filtrate was concentrated and purified by silica gel chromatography. To hydrolyze the

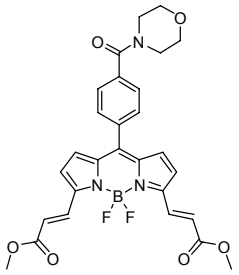
tert-butyl ester BF_3OEt_2 (0.3 mL) was added at 0 ^oC to the solution of ester in DCM (70 mL). After stirring for 1hr, the reaction mixture was diluted with DCM and acidified to pH 3 with aq. HCl solution. The aqueous layer was extracted with 5% *i*PrOH / DCM five times. The organic layer was dried over sodium sulfate. The filtrate was concentrate and purified by silica gel chromatography (MeOH : DCM : H_2O = 10 : 50 : 1) to give **4a** (60 mg, 80%) ^{1}H-NMR (400 MHz, $CDCl_3$+ CD_3OD) δ 8.11 (d, *J* = 16.0, 2H), 7.58 (m, 5H, Ph), 7.04 (d, *J* = 4.0, 2H), 6.96 (d, *J* = 4.0, 2H), 6.66 (d, J = 16.0, 2H); ^{13}C-NMR ($CDCl_3$+ CD_3OD) 167.88, 131.98, 131.62, 131.41, 130.80, 130.17, 128.47, 128.29, 128.11, 127.35, 126.01, 125.35 ; ESI-HRMS *m/z* $(M+Na)^+$ calc'd: 431.0985, found 431.0990

(4-(di(1H-pyrrol-2-yl)methyl)phenyl)(morpholino)methanone (1b): To a solution of 4-(morpholine-4-carbonyl)benzaldehyde (220 mg, 1.0 mmol) in DCM (10 ml), pyrrole (2.2 mmol) was added. The mixture was blown under nitrogen for 10 min. TFA (0.1 mmol) was added. The reaction mixture was stirred at room temperature for 4 hrs. The reaction was quenched with 0.2 *N* NaOH aqueous solution (20 ml) and extracted with EtOAc. The organic layer was washed with brine and dried over sodium sulfate. The filtrate was concentrated and purified by silica gel chromatography (DCM: EtOAc =8: 1) to give **1b** (108 mg, 32%). 1H-NMR (300 MHz, $CDCl_3$) δ 8.06 (bs, 2H, 2-NH), 7.35 (d, *J* = 8.4, 2H, 2-CH), 7.26 (d, *J* = 8.4, 2H, 2-CH), 6.70 (dd, *J* = 1.8, 4.2, 2H, 2-CH), 6.16 (dd, *J* = 3.0, 6.0, 2H, 2-CH), 5.90 (bs, 2H, 2-CH), 5.49 (s, 1H, -CH), 3.71 (bt, 8H, 4-CH2-); ^{13}C-NMR (CDCl3) δ 170.2, 144.20, 131.70, 128.53, 127.63, 127.41, 117.42, 108.44, 107.32, 66.83, 43.78, 38.66. ESI-MS *m/z* $(M+H)^+$ calc'd: 336.2, found 336.1



5,5'-((4-(morpholine-4-carbonyl)phenyl)methylene)bis(1H-pyrrole-2-carbaldehyde) (2b): To DMF (1 mL) was added $POCl_3$ (150 µL, 1.60 mmol) slowly, and the mixture was stirred for 5 min at 0 ^oC. This Vilsmeier reagent (750 mL, 1.10 mmol) was added slowly to a solution of **1b** (150 mg, 0.45 mmol) in DMF (1.50 mL), and stirred for 1.5 hr at 0 ^oC. The saturated sodium acetate solution (5 mL) was added and stirred at RT overnight. After the reaction completion monitored by TLC, the reaction mixture was diluted with EtOAc, and washed with water and brine. The organic layer was dried over sodium sulfate. The filtrate was concentrated and purified by silica gel column chromatography (DCM: EtOAc = 5 : 1) to give the greenish yellow solid (142 g, 81%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.67 (bs, 2H, 2-NH), 9.32 (s, 2H, 2-CHO), 7.34 (d, *J* = 8.4, 2H, 2-CH), 7.24 (d, *J* = 8.4, 2H, 2-CH), 6.90 (dd, *J* = 1.8, 4.2, 2H, 2-CH), 6.09 (dd, *J* = 2.4, 3.2, 2H, 2-CH), 5.60 (s, 1H, -CH), 3.72 (bt, 8H, 4-CH2-); ^{13}C-NMR ($CDCl_3$) δ 176.09, 163.61, 146.38, 137.67, 131.53, 130.60 129.22, 128.36, 128.33, 123.08, 111.92, 67.45, 54.09, 39.24; ESI-MS *m/z* $(M+H)^+$ calc'd: 391.2, found 391.1





Bodipy diacrylate 4b: To a solution of **2b** (40 mg, 0.10 mmol) in DCM (1 mL) was added methoxycarbonylmethylene triphenylphosphorane (100 mg, 0.30 mmol) at 0^{o}C. After stirring at RT overnight, the reaction mixture was diluted with DCM and washed with brine. The organic layer was dried over sodium sulfate. The filtrate was concentrated and purified by silica gel column chromatography (DCM : EtOAc = 8 : 1) to give a greenish yellow solid (36 mg, 71%); This yellow solid (36 mg, 0.07 mmol) was dissolved in DCM (7 mL). To this solution was added DDQ (24 mg, 0.11 mmol). After stirring for 15 min at RT, the reaction mixture was cooled to 0^{o}C. To this solution, DIEA (295 uL, 1.75 mmol) and BF_3OEt_2 (225 uL, 1.75 mmol) were added and slowly warmed up to RT while stirring for 2 hrs. The reaction mixture was diluted with DCM, and washed with sat. $NaHCO_3$ and brine. The organic layer was dried over sodium sulfate. The filtrate was concentrated and purified by silica gel chromatography (DCM : EtOAc = 10 : 1) to give **4b** (24 mg, 62%) ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.14 (d, *J* = 15.9, 2H, 2-CH), 7.55 (m, 4H, -Ph), 6.91 (d, *J* = 4.5, 2H, 2-CH), 6.87 (d, *J* = 4.5, 2H, 2-CH), 6.60 (d, *J* = 15.9, 2H, 2-CH), 3.87 (s, 6H, 2-CH3), 3.84 (bt, 4H, 2-CH2-), 3.80 (bt, 4H, 2-CH2-); ^{13}C-NMR ($CDCl_3$) 169.14, 166.14, 132.95, 132.15, 132.02, 131.99, 131.57, 130.67, 128.60, 128.44, 127.39, 125.53, 66.86, 58.19, 29.70; ESI-HRMS *m/z* $(M+Na)^+$ calc'd: 572.1780, found 572.1786.

2. Peptides (P1-P6) Preparation

Peptides were synthesized on Rink Amide MBHA resin with standard Fmoc-protected amino acids/HBTU coupling steps followed by piperidine deprotection [Coupling step conditions: Resin (100 mg, 0.48 mmol/g), 0.5 M HBTU in DMF (0.6 mL), 0.5 M Fmoc-Amino acid in NMP (0.6 mL) and 2.0 M DIEA in NMP (0.42 mL) for 3.5 hrs; Deprotection condition: 20% piperidine in NMP (1.5 mL) for 1 hr]. The final N-terminal was capped by acetylation (0.3 M of Ac_2O and 0.27 M of HOBt in DCM for 2hrs). Peptides were deprotected and cleaved from the resin with the reagent K solution (TFA : H_2O : thioanisole : phenol : EDT = 10 mL : 0.5 mL : 0.5 mL : 0.75 g : 0.25 mL). Each cleavage solution was drained to chilled ether (20 mL) to

precipitate the peptide. Peptide solutions were kept at -20 ^{o}C overnight for the maximal precipitation. The precipitates were filtered and dried. Peptides were purified by reverse phase HPLC on C18 preparative column with a linear gradient from 0 ~ 50% acetonitrile in H_2O containing 0.1% TFA. The collected peptide solutions were lyophilized and the peptide solids were kept at -20 ^{o}C. Purity was determined by LC-MS with condition of two different column C18, 4.6 x 50 mm and C18 4.6 x 150 mm with different eluents composition at the wavelength of 214 nm.

3. Calculation process to determine the reaction rate constant between dye and peptide.

As peptide is large excess, the concentration of peptide was considered to be constant during the reaction with dye. The reaction between dye and peptide was considered as *pseudo*-first order reaction.

$r = k[dye][peptide] = k'[dye] = -d[dye] / dt$
$d[dye] / [dye] = -k'dt$
$\ln[dye] = -k't + \ln[dye]_0$
$\ln([dye]_0 / [dye]) = k't$

as $[dye]_0 / [dye] = [product]_\infty / ([product]_\infty - [product])$
$= F_\infty / (F_\infty - F_t)$

so if we plot $\ln(F_\infty / (F_\infty - F_t))$ over time t, the slope value is k', which is the *pseudo*-first order reaction rate constant.
[dye]: dye concentration as time t
$[dye]_0$: dye initial concentration
[product]: product concentration at time t
$[product]_\infty$: product final concentration
F_∞: fluorescent intensity of product (530 nm) at saturation.
F_t: fluorescent intensity of product (530 nm) at time t

4. Construction of the expression vectors

pc-**RC•**myc: An oligonucleotide (**RC•** myc) encoding the RC tag (P1 peptide, written in green) together with the Myc-tag (blue) was synthesized (GGGGCTAGCCCACCATGGAA GCTGCCGCACGTGAAGCGAGATGTCGTGAGCGCTGCGCGAGAGA AGCTTGAACAAAAACTCATCTCAGAAGAGGATCTGGGATCCCC, restriction enzyme sites inserted for cloning are underlined). Nucleotides marked in green encode the RC tag and in blue encode the Myc tag. Myc tag was inserted to be used as the epitope in the western blotting for the confirmation of recombinant protein expression. Using the "**RC•** myc" as the template, PCR was performed with two short primers (GGGGCTAGCCCACCATGGAA +GGGGGATCCCAGATCCTCTTC). The resulting PCR product was digested with NheI/BamHI and subcloned into the NheI/BamHI sites of pcDNA3.1(+) (Invitrogen). This clone was named as pc-**RC•**myc and was used for further subclonings.

pc-**RC•**myc•Cherry: We amplified the open reading frame (ORF) of a red fluorescent protein (mCherry) using the primers (GCTGGATCCATGGTGAGCAAGGGCGAGGAGGACAA CATG+GGGCTCGAGTCACTTGTACAGCTCGTCCATGCCGCCGGT GGA) using the pc-mCherry (Clontech) as the template and inserted into the BamHI/XhoI sites of pc-**RC•** myc. The resulting plasmid (pc-**RC•**myc•Cherry) expresses the monomeric Cherry that is tagged with the P1 peptide and the Myc-tag.

RC tag mutant clones (**m1, m2, m3**): Vectors express mCherry fused to three mutant tags were prepared by site-directed mutagenesis PCR. Oligonucleotide sets encoding arginine in the place of cysteine are designed as below.

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m1_S  : GCACGTGAAGCGAGAGCTCGTGAGCGCTGCGCG
m1_AS: CGCGCAGCGCTCACGAGCTCTCGCTTCACGTGC
m2_S  : AGATGTCGTGAGCGCGCCGCGAGAGCTAAG
m2_AS: CTTAGCTCTCGCGGCGCGCTCACGACATCT
m3_S  : AGAGCTCGTGAGCGCGCCGCGAGAGCTAAg
m3_AS: CTTAGCTCTCGCGGCGCGCTCACGAGCTCT
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25ng of pc-**RC•**myc•Cherry was used as the template for mutations and the PCR-reactions were performed with pfu DNA polymerase with a cycling profile of 95 ºC 30 sec, (95 ºC 30 sec, 55 ºC 60 sec, 68 ºC 10 min) x 16 cycles. Reaction product was digested with DpnI for 1 hour and transformed to *E. coli* strain DH5. Acquired mutant clones were confirmed by nucleotide sequencing and designated as pc-(**m1**)**RC•** myc•Cherry, pc-(**m2**)**RC•** myc•Cherry, pc-(**m3**)**RC•** myc•Cherry, respectively.

pc-**RC^2•**myc•Cherry: Oligonucleotides encoding the RC tag with the Myc tag but without Kozak or initiating methionine codon (ATG) was synthesized (RC^2•myc: CAAGCTTGAAGCTGCCGCACGTGAAGCGAGATGTCGTGAGCGCT GCGCGAGAGCTGAATTCGCCGATATCGAACAAAAACTCATCTCA GAAGAGGATCTGGGATCCC). Using the "RC^2• myc" as the PCR template, the "RC^2• myc" was amplified with primers (CCCAAGCTTGAAGCTGCCGCA+GGGGGATCCCAGATCCTCTTC). The resulting PCR product was digested with HindIII/ BamHI and inserted into the HindIII/BamHI sites of the pc-pc-**RC•**myc•Cherry. The acquired clone has a dimerized **RC** tag and a myc epitope fused to the Cherry. The amino acid sequence encoded by the resulting **RC^2** is shown below.

RC : MEAAAREARCRERCARA
RC^2 : MEAAAREARCRERCARAKLEAAAREARCRERCARA

pc-**RC^2•**(NLS)•myc•Cherry: An oligonucleotide encoding triple copies of nuclear localization signal of the SV40 Large T antigen[S2] was synthesized as below. The oligonucleotides were hybridized in the Tris-buffer (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9) by boiling and slowly cooling-down to the room temperature and digested with EcoRI/EcoRV. The digested double stranded DNA fragment was inserted into the

EcoRI/EcoRV sites of pc-**RC^2•**myc**•**Cherry and the resulting clone was named as pc-**RC^2•**(NLS)**•**myc**•**Cherry.
3xNLS (S):CCCGAATTCGATCCCAAAAAGAAACGCAAGGTGG
ATGATCCCAAAAAGAAACGCAAGGTGGATGATCCCAAAAAGAAA
CGCAAGGTGGATATCGGG
3xNLS(AS):CCCGATATCCACCTTGCGTTTCTTTTTGGGATCA
TCCACCTTGCGTTTCTTTTTGGGATCATCCACCTTGCGTTTCTT
TTTGGGATCGAATTCGGG

pc-**RC^2•**myc**•**H2B: ORF of human histone H2B was PCR amplified using the cDNA of normal human fibroblasts. Primers used the amplification were (GGGGGATCCATGCCT
GAACCGGCAAAATC+GGGCTCGAGTCACTTGGAGCTGGTGTACT) and the resulting PCR product was digested with BamHI/XhoI and subcloned into the BamHI/XhoI sites of pc-**RC^2•** myc**•**Cherry to exchange the ORF of Cherry with that of H2B.

pc-**RC^2•**myc**•**H2B**•**Cherry: The ORF of human H2B was PCR-amplified with primers (GGGGAATTCATGCCTGAACCGG
GCAAAATC+GGGGATATCCTTGGAGCTGGTGTACTTGG) and the PCR product was digested with EcoRI/EcoRV. Digested DNA was inserted into the EcoRI/EcoRV sites of the pc-**RC^2•**(NLS)**•**myc**•**Cherry to exchange the NLS with the H2B ORF.

pc-**RC•**Cherry: ORF of Cherry was amplified with primers (GGGAAGCTTATGGTGAGCAAGGGCGAGGAG+GGGCTCGAGCTT
GTACAGCTCGTCCATGCCGCCGGTGGA) and the resulting PCR product was digested with HindIII/XhoI and introduced into the HindIII/XhoI sites of pc-**RC.**myc**.**Cherry to generate the pc-**RC•**Cherry.

pc-6xHis**•**myc**•RC•**Cherry: Primers GGGCTAGCCACCATGCAT
CATCATCATCACCACGAATTCGAACAAAAACTCACTCAGAA+CC
CAAGCTTAGCTCTCGCGCAGCGCTCACG) was used to amplify the expression cassette of "6xHis tag and RC tag". The produced PCR product was digested with Nhe1/HindIII and inserted into the NheI/HindIII site of pc-**RC•**myc**•**Cherry.

pc-Cherry**•**myc**•RC**: Primers (GGGAATTCGAACAAAAACTCAT
CTCAGAAGAGGATCTGGATATCGAAGCTGCCGCACGTGAA+CCC
CTCGAGTCAAGCTCTCGCGCAGCGCTC) were used to amplify the "myc tag-RC tag" cassette and resulting PCR product was digested with EcoR1/Xho1 and cloned into the pcDNA3.1(+), resulting the pc-myc**•RC**. ORF of Cherry was PCR amplified with (GGGGCTAGCCACCATGGTGAGCAA
GGGCGAGGAG+GGGGAATTCCTTGTACAGCTCTCCATGCCGCCG
GTGGA) and the acquired PCR product was cloned into the NheI/EcoRI sites of the pc-myc**•RC** resulting the pc-Cherry**•**myc**•RC**.

5. Cell culture and transfection

HEK293 cells, an immortalized line of primary human embryonic kidney cells, were purchased from Invitrogen and maintained in the DMEM (10% Fetal Bovine Serum (FBS), 1% antibiotics-antimycotics reagent). Materials used in the cell culture were purchased from Invitrogen. For transient transfection, cells were plated at the density of $2x10^5$ cells/well in 12 well plate and 500 ng of plasmid DNA purified by Midi-prep kit (Qiagen) were transfected with Lipofectamine 2000 (Invitrogen). After 2 days incubation, the transfected cells were subjected to the following experiment such as live cell staining or (SDS-PAGE /western blotting).

6. SDS-PAGE, gel-scanning, western blotting, and silver staining Total protein was extracted by using CelLyticM^{TM} cell lysis solution (Sigma). Generally 10μg of the protein/well was loaded in SDS-PAGE gel for gel-scanning. NuPAGE Novex Bis-Tris Gels (Invitrogen) were used for PAGE and the gel was scanned using the Typhoon 9410 Gel Scanner (GE Healthcare). Gel was excited at stained 488nm and was scanned through 526SP emission filter. After gel scanning, proteins were transferred onto the PVDF membrane and subjected to the following western blotting. Western blotting data were generated by fluorescence scanning of the membranes stained with antibodies. A mouse monoclonal α-myc (Santa Cruz, sc-40) antibody and a goat α-mouse IgG tagged with Cy5 (Invitrogen, A10524) were used. Membranes were excited at 633nm and scanned through 670BP emission filter. When the gel was subjected to the silver staining, gel was fixed in fixing solution (50% EtOH, 10% glacial acetic acid) for 10 min. Gel was rinsed with water for 1hr and then, sensitized in 0.02% $Na_2S_2O_3$ for 2 min. After a brief rinsing with water, gel was stained in 0.1% $AgNO_3$ for 30 min. After rinsing with water, gel was developed with 2% Na_2CO_3, 37% (v/v) formaldehyde and the reaction was stopped with 1% CH_3COOH.

7. Compound staining and imaging in the live cells Compounds were reconstituted in DMSO as the 1 mM solution, and stored in -20 ºC. Immediately before staining, medium in the wells were drained and the compound diluted in the pre-warmed growth medium was added directly onto the cells. After incubation (30 min, 37 ºC), cells were washed with growth medium and further incubated in the cell culture incubator for 1 hour. Medium was changed once again, and cells were subjected to the live cell imaging. Bright field images and fluorescence images were acquired by a fluorescent microscope ECLIPSE Ti-E (Nikon Instrument Inc.). Emission filters used are DAPI filter (Ex 340-380nm, Em 435-485nm) for Hoechst, FITC filter (Ex 465-495nm, Em 515-555nm) for **4b**, and Cy5 filter (Ex 590-650nm, Em 663-738nm) for Cherry.

References

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[S2] D. Kalderon, B. L. Roberts, W. D. Richardson and A. E. Smith, *Cell*, 1984, **39**, 499-509.

Supplemental Figures

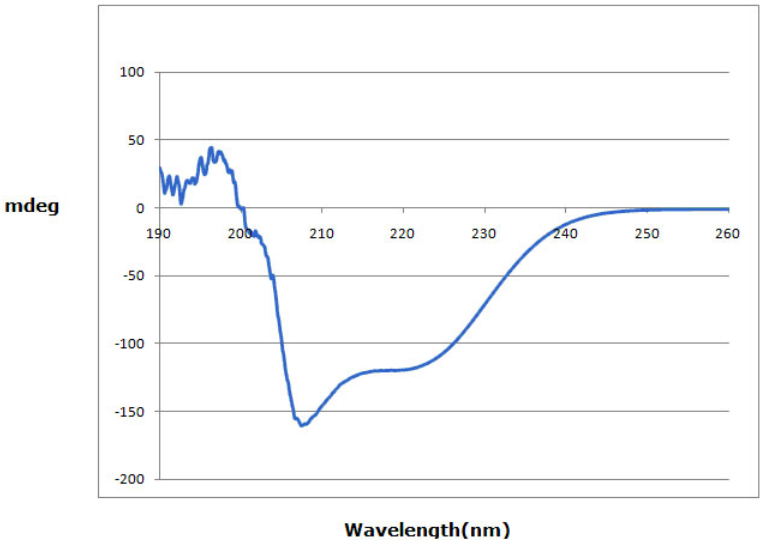
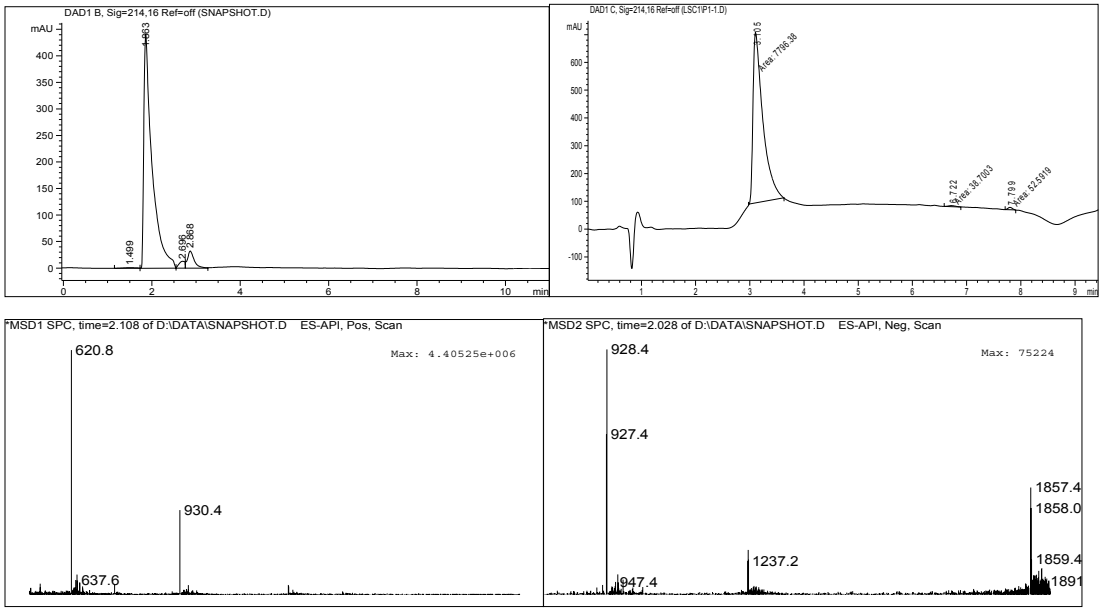


Fig. S1. Circular Dichroism (CD) spectrum of P1. CD was determined with 1mM of **P1** in 10mM PBS buffer solution. Buffer reading value was subtracted. Typical helix 209, 220 nm excitations were observed.

P1: AcEAAAREARCRERCARA-NH_2

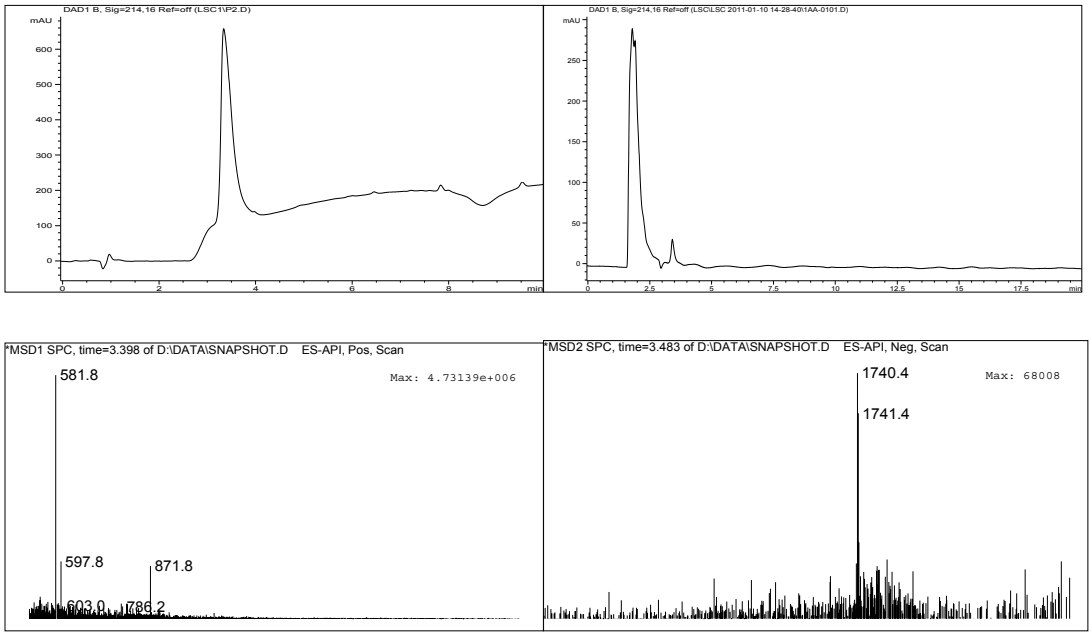
a b



a. ESI-Mass : calculated:1858.9, found; 930.4$(M + 2H)^{2+}$,620.8$(M + 3H)^{3+}$,1857.4$(M - H)^{-}$

P2, AcEAAAREAAARERCARA-NH_2

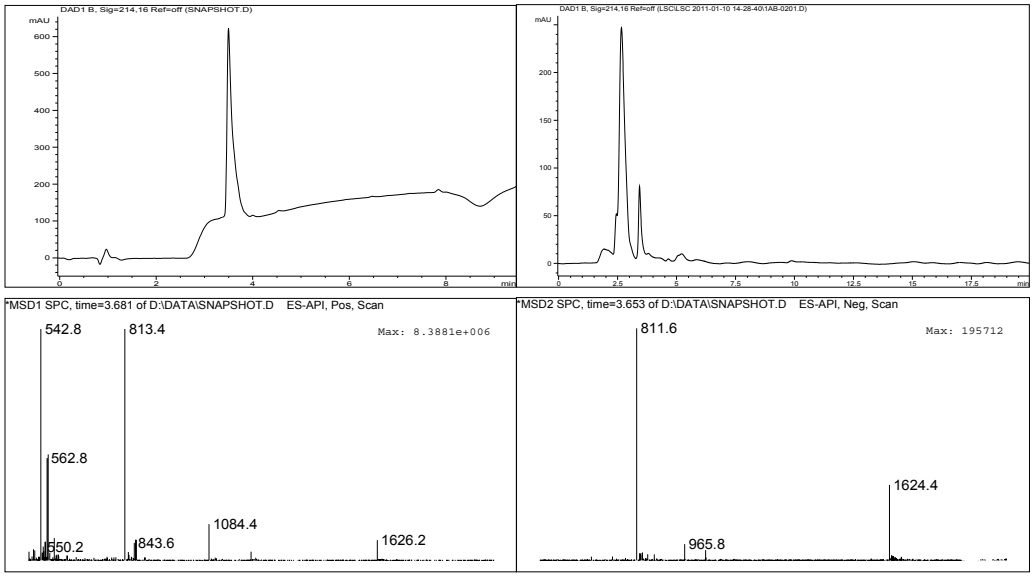
a b



c. ESI-Mass: calculated; 1741.9, found; 871.8$(M + 2H)^{2+}$, 581.8$(M + 3H)^{3+}$, 1740.4$(M - H)^{-}$

P3, AcEAAAREAAAREAAARA-NH_2

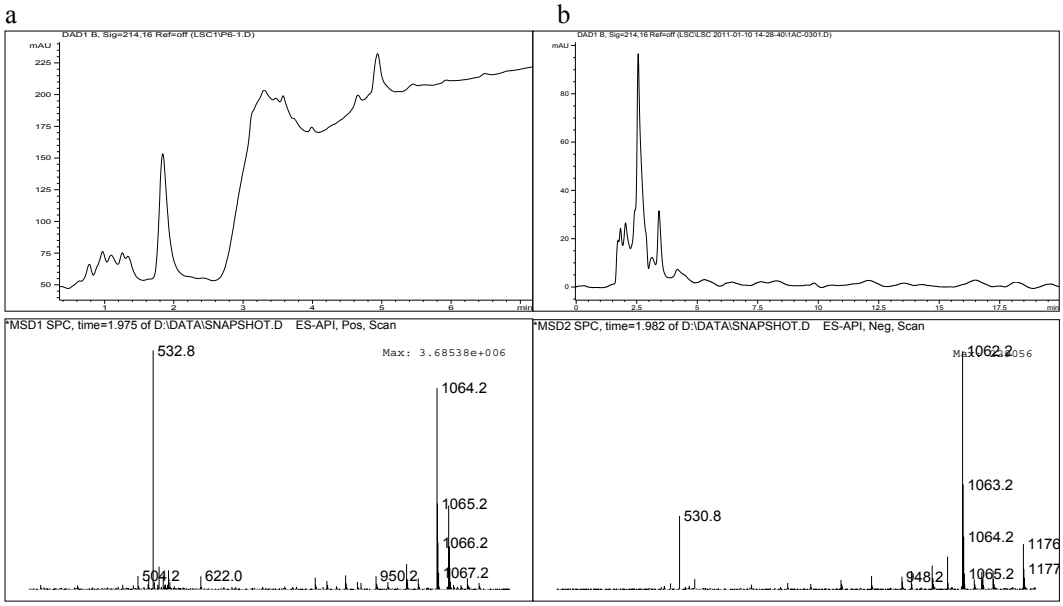
a b



c. ESI-Mass: calculated; 1624.8, found; 1626.2$(M + H)^+$, 813.4$(M + 2H)^{2+}$,542.8$(M + H)^{3+}$,1624.4$(M - H)^-$

P6, AcGGGGGGGGCGGGCGGG-NH_2

a b



C ESI-Mass: calculated; 1063.4, found; 1064.2$(M + H)^+$, 532.8$(M + 2H)^{2+}$, 1062.3$(M - H)^-$

Fig. S2 LC-MS chromatogram of P1, P2, P3, P6 peptides. LC condition of a: (5% ACN to 100% ACN gradient condition with water, contained 0.1% TFA, run time :10min, column: C18, 4.6 x50 mm, 5micron, monitored at 214 nm channel) LC condition of b: (15% ACN in water isocratic, contained 0.1% TFA, run time :20min, column: C18, 4.6 x 15 mm, 5micron, monitored at 214 nm channel)

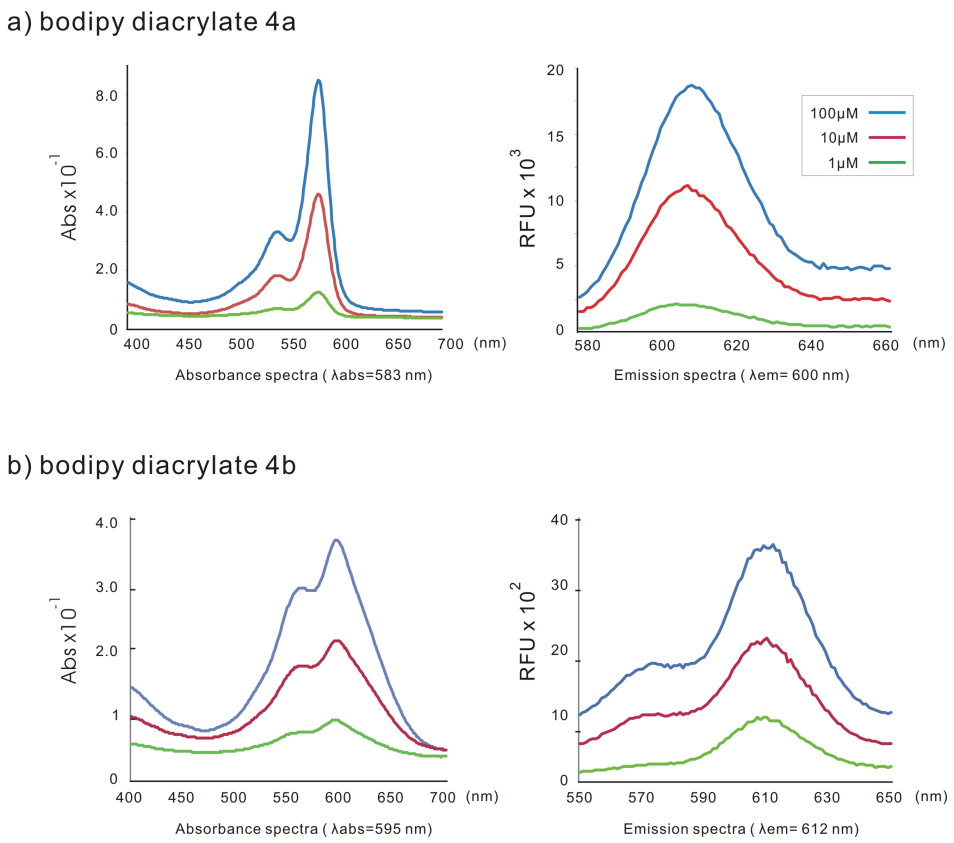
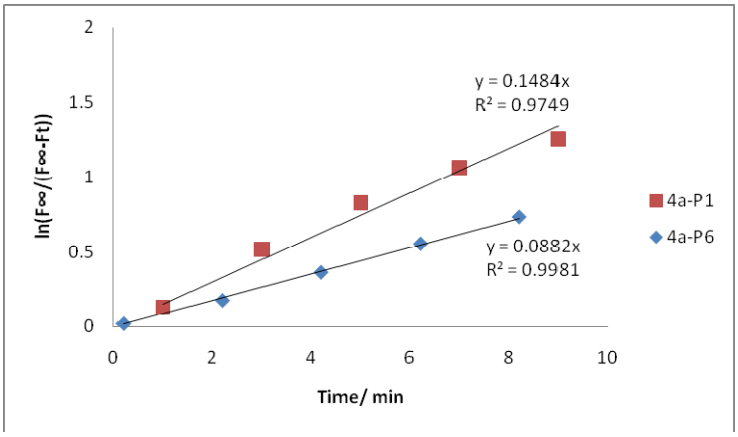


Fig S3. Spectral properties of 4a and 4b. (a) Absorbance and emission spectra of **4a** in 50mM HEPES buffer (pH 7.4). Fluorescence spectra were obtained with an excitation at 550 nm (Absorption coefficient; = 66,888, Quantum Yield; = 0.29 over 1,3,5,7-Tetramethyl-8-phenyl BODIPY as a reference). (b) Absorbance and emission spectra of **4b** in 50mM HEPES buffer (pH 7.4). Fluorescence spectra were obtained with an excitation at 520 nm (Absorption coefficient; = 55,989, Quantum Yield; = 0.21 over 1, 3, 5, 7-Tetramethyl-8-phenyl BODIPY as a reference).



Linear plot for the **k** value determination

Reactions	constant k (s^{-1})	R^2
4a-P1	0.1484	0.9749
4a-P6*	0.0882	0.9981

Fig S4. Kinetic study to determine the reaction rate constant between dye and peptide. 10 μM of **4a** and 200 μM of peptide (P1 or P6*) of 20 mM HEPES buffer solution, containing 1 % DMSO, was placed in a 96-well plate. Fluorescent intensity at 530 nm, with an excitation at 480 nm, was recorded every 2 min until saturated under a xenon flash lump.(control peptide without Arg. **P6*:** AcGGGGGGGG**C**GGG**C**GGG-NH_2)

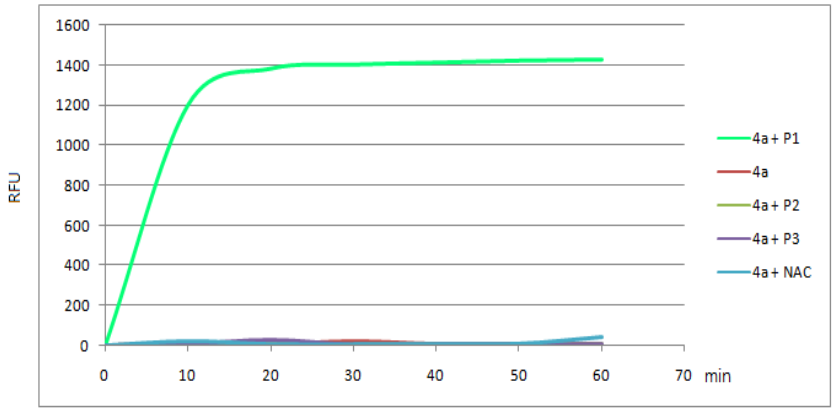
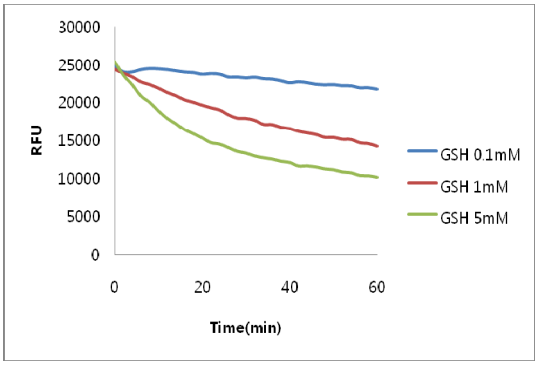
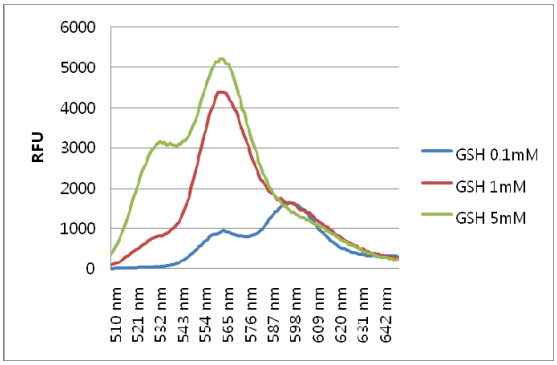


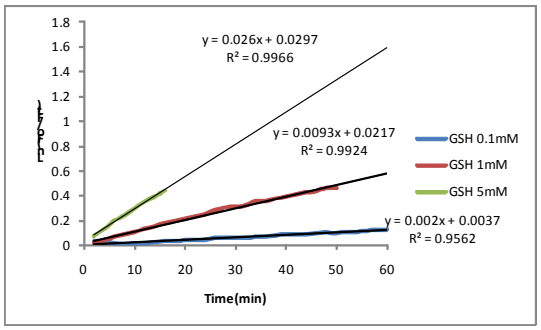
Fig S5. **Time-dependent fluorescence responses of 4a incubated with model peptides**: **4a** (10 μM) was mixed with P1, P2, P3 (10 μM) or NAC (100 μM) in 50mM HEPES (pH 7.4) and the fluorescence emissions were measured at 530 nm with an excitation at 480 nm.



a. Decrease of fluorescence intensity at 600 nm



b. After 40 min incubation with GSH, full spectrum of 4a



c. Linear plot for the **k** value determination

Fig. S6. Fluorescence responses of 4a incubated with various concnetration of glutathione. (a) Glutathione was reacted with **4a** (10 μM) in 50 mM HEPES (pH 7.4) with an excitation at 550 nm and the emission spectra acquired at 2 min intervals. (b) Glutathione was reacted with **4a** (10 μM) in 50 mM HEPES (pH 7.4) with an excitation at 480 nm and the emission spectra are acquired after 40 min incubation at RT. (c) Calculation of rate constant; As GSH is large excess, the concentration of GSH was considered to be constant during the reaction with **4a**. The reaction between dye and GSH was considered as *pseudo*-first order reaction.

$r = k[dye][GSH] = k'[dye] = -d[dye] / dt$

$d[dye] / [dye] = -k'dt$

$\ln[dye] = -k't + \ln[dye]_0$

$\ln([dye]_0 / [dye]) = k't$

as $[dye]_0 / [dye] = F_0 / F_t$

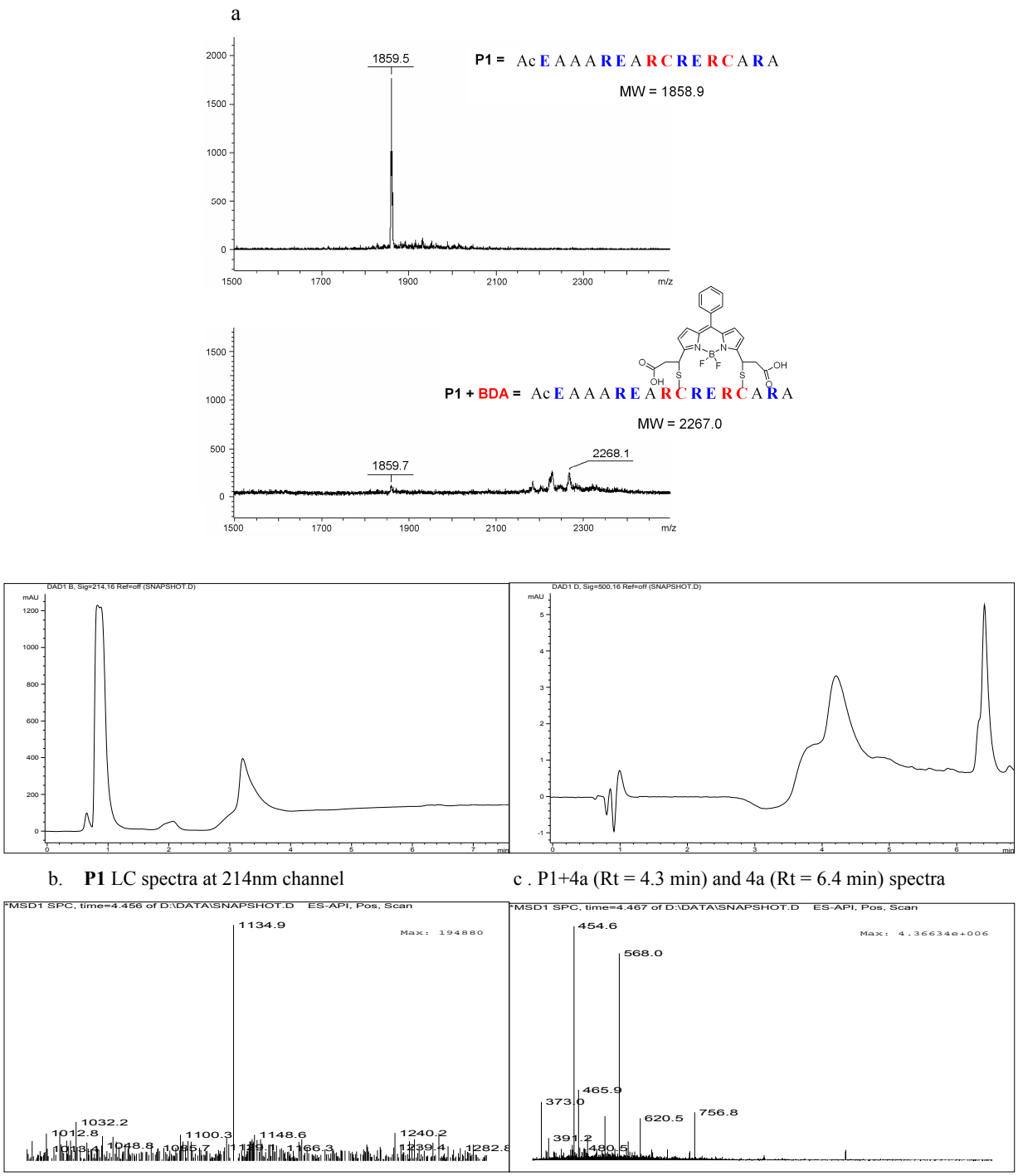
so if we plot $\ln(F_0 / F_t)$ over time t, the slope value is k', which is the *pseudo*-first order reaction rate constant.

[dye]: dye concentration as time t

$[dye]_0$: dye initial concentration

F_0: initial fluorescent intensity of dye (600 nm).

F_t: fluorescent intensity of dye (600 nm) at time t



d. ESI-mass found of P1 + 4a conjugation: 1134.9$[M + 2H]^{2+}$, 756.8$[M + 3H]^{3+}$,568.0$[M + 4H]^{4+}$, 454.6$[M + 5H]^{5+}$

Fig. S7. Conjugation of 4a to peptide P1. (a) MALDI-TOF spectra of a model peptide P1 after incubation with **4a: 4a** (10 μM) and P1 (20 μM) were mixed in 50 mM HEPES (pH 7.0), then mixture was analyzed after desalting by C18 ziptip. Mass indicates the presence of the conjugation product of P1-**4a** (2268.1, M+H), with another mass peak at 2228.9 (M-38). (b-d) LC-MS chromatogram shift after conjugation with P1 and **4a**. Original P1 peptide (Rt = 3.3min) was shifted to 4.3 at 500nm UV channel. Multiple charged mass was observed at the shifted peak.

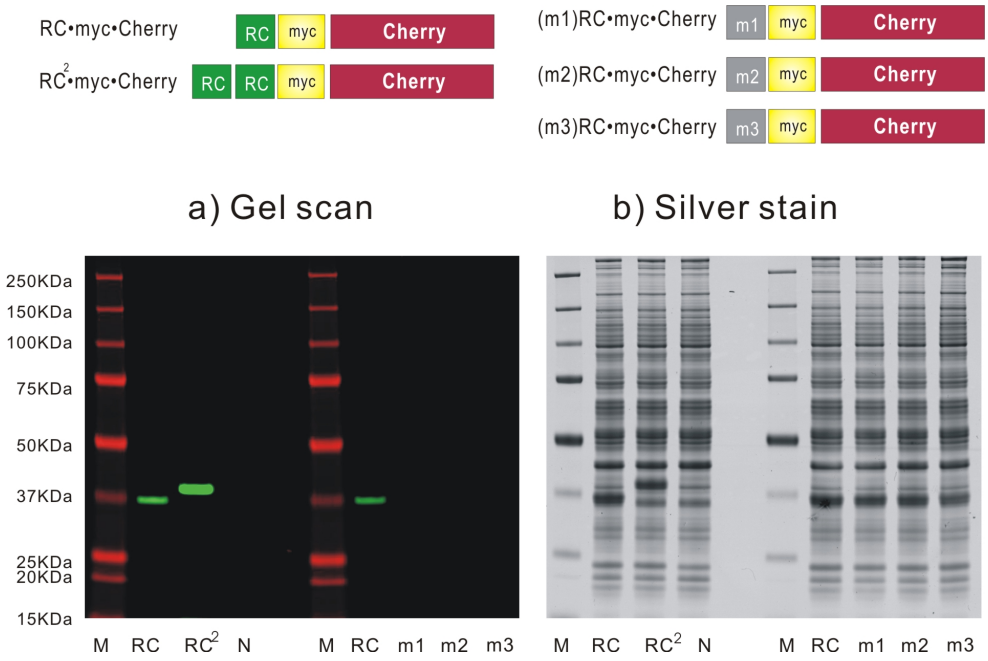


Fig. S8. Specific-conjugation of 4b to RC^2 tagged target proteins in the total proteome. HEK293 cells, transfected with the **RC**·myc·Cherry and **RC**^{2}·myc·Cherry or the alanine mutation clones, were stained with **4b** (1μM, 30 min, 37ºC) and the total lysates were analyzed on SDS-PAGE. After fluorescence gel scanning (a), the gel was subjected to silver staining (b) to reveal the total proteome resolved on gel.

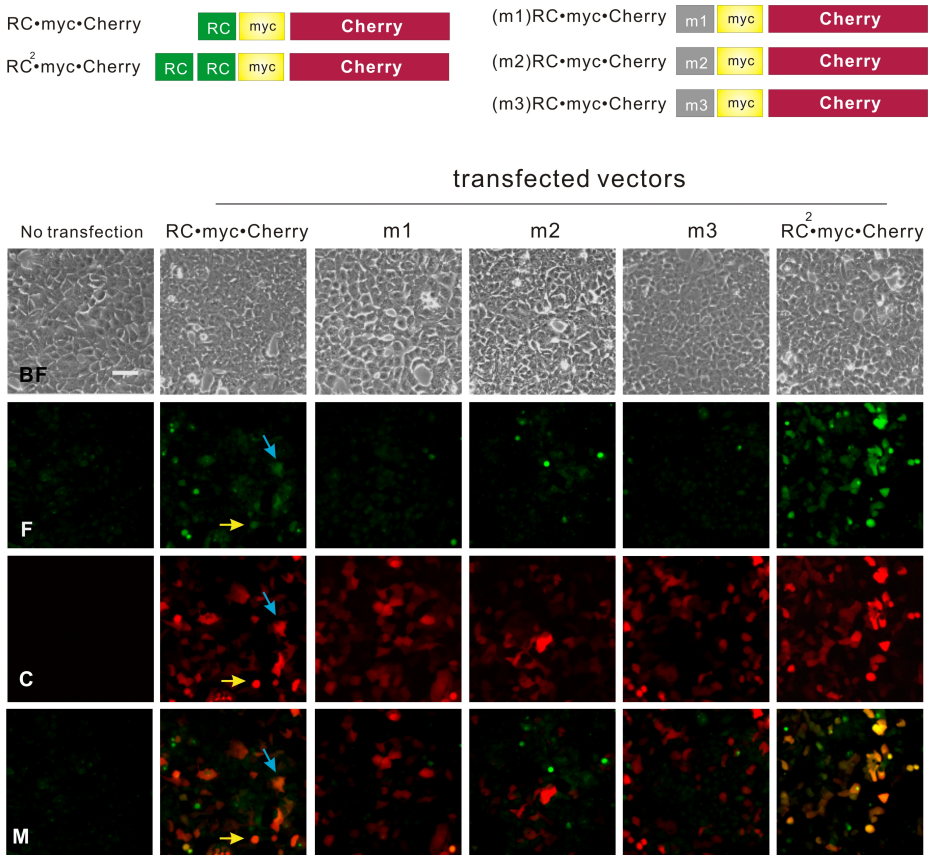


Fig. S9. Dimerization of RC tag enables an effective labeling of target protein by 4b in live cells. HEK 293 cells were transfected with the expression vectors encoding Cherry that is tagged with **RC**, **RC**^{2} or the alanine mutants of RC (m1, m2, m3) and stained with 4b. Fluorescence microscopic images taken by FITC filter (F) show the green fluorescence resulting from the spectral change of compound and images taken by Cy5 filter (C) prove the expression of tagged protein (Cherry). The yellow and blue arrows in **RC** tagged Cherry (**RC**• myc• Cherry) indicate cells showing overlapping signals (green and red). Noticeably, the green signal from the dimeric RC tagged protein (**RC**^{2}• myc• Cherry) is significantly stronger than the monomeric RC tagged protein (**RC**• myc• Cherry). BF-bright field, F-FITC, C-Cy5. M-merged (F and C), Scale bar - 50µm.

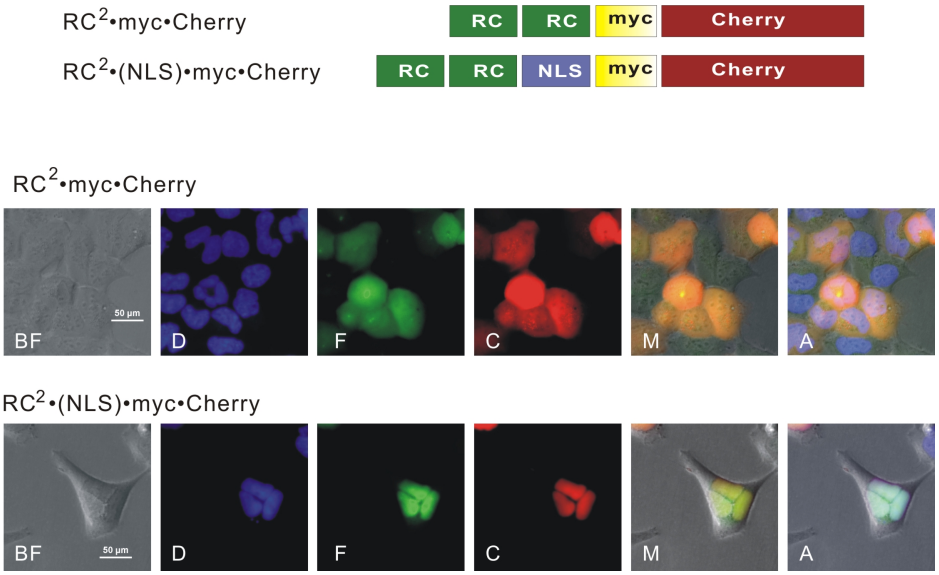


Fig. S10. Fluorescence microscopic images of RC^2 tagged nuclear protein labeled by 4b in HEK293 cells. Cells transfected with the pc-**RC^2**•myc•Cherry or pc-**RC^2**•(NLS)•myc•Cherry and were stained with **4b** (1μM, 15 min, 37ºC) and followed by the Hoechst staining (10μM, 30 min, 37ºC). Images were taken in live cells. Filters used for fluorescence imaging were BF-bright field, D-DAPI, F-FITC, C-Cy5, M-Merged., A- images of all filters are merged (BF+D+F+C). scale bars - 50μm.

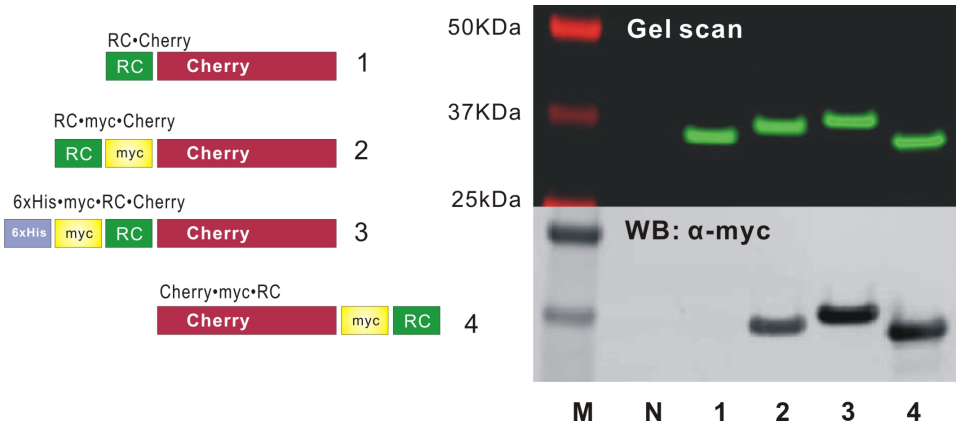
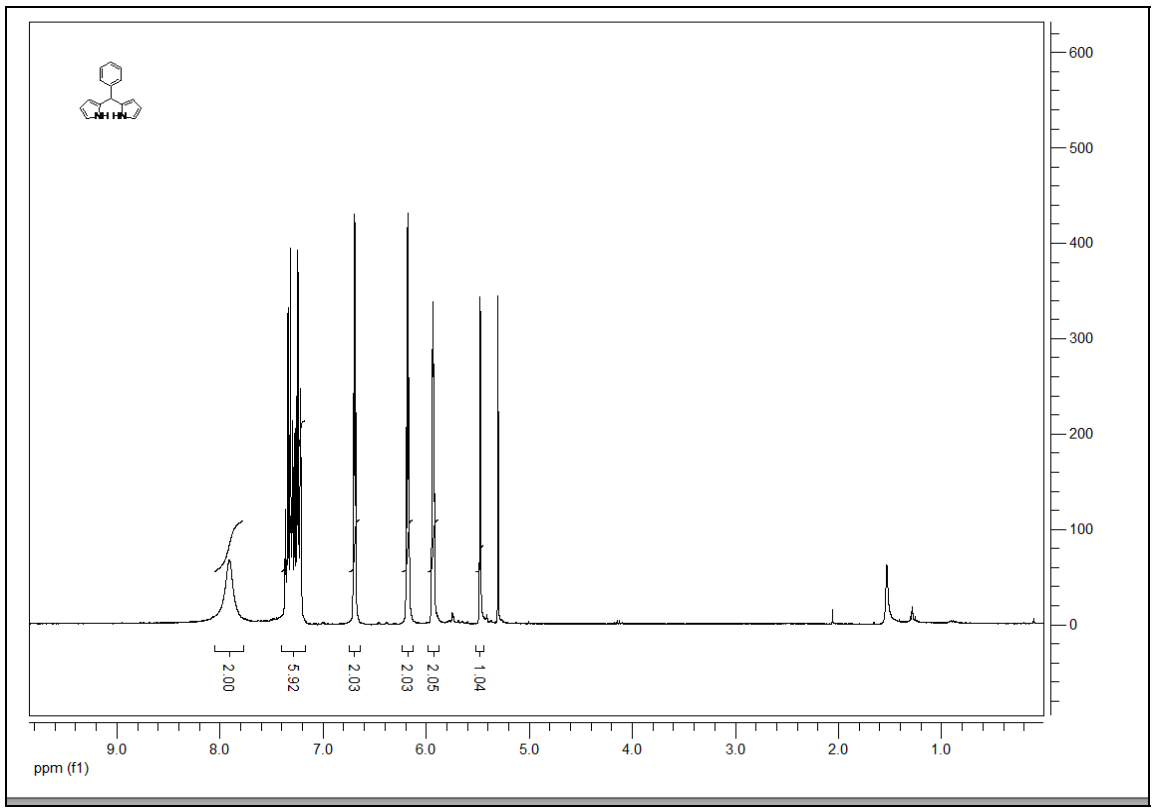
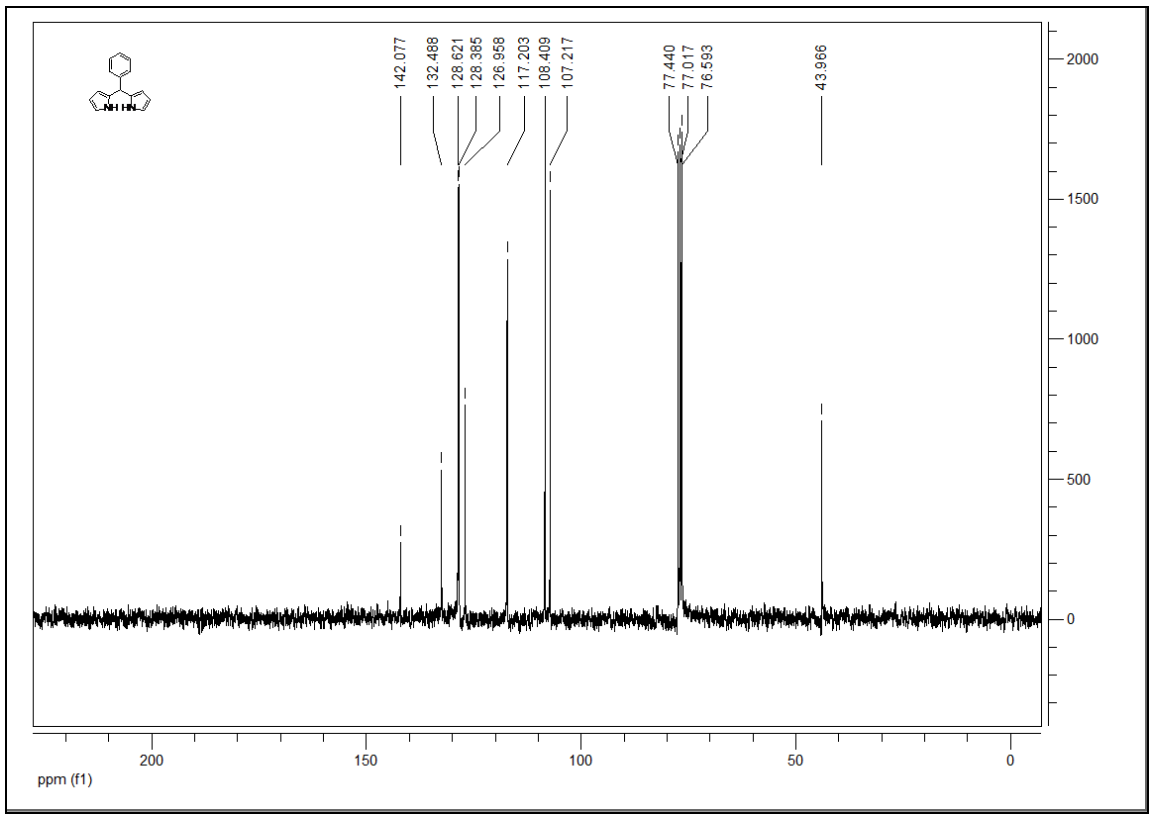


Fig. S11. Labeling of target protein by RC tag in various location. Plasmid vectors were prepared to express the monomeric Cherry with an **RC** tag placed in diverse position in combination with other small peptide tags. HEK293 cells transfected with the expression vectors were stained with **4b** (1μM, 30 min, 37ºC) and the total lysates were analyzed on SDS-PAGE. After fluorescence scanning of the gel, protein was transferred to PVDF membrane and was subjected to western blotting (α-myc) for the confirmation exogenous protein expression. M-protein size marker, N-no transfection.

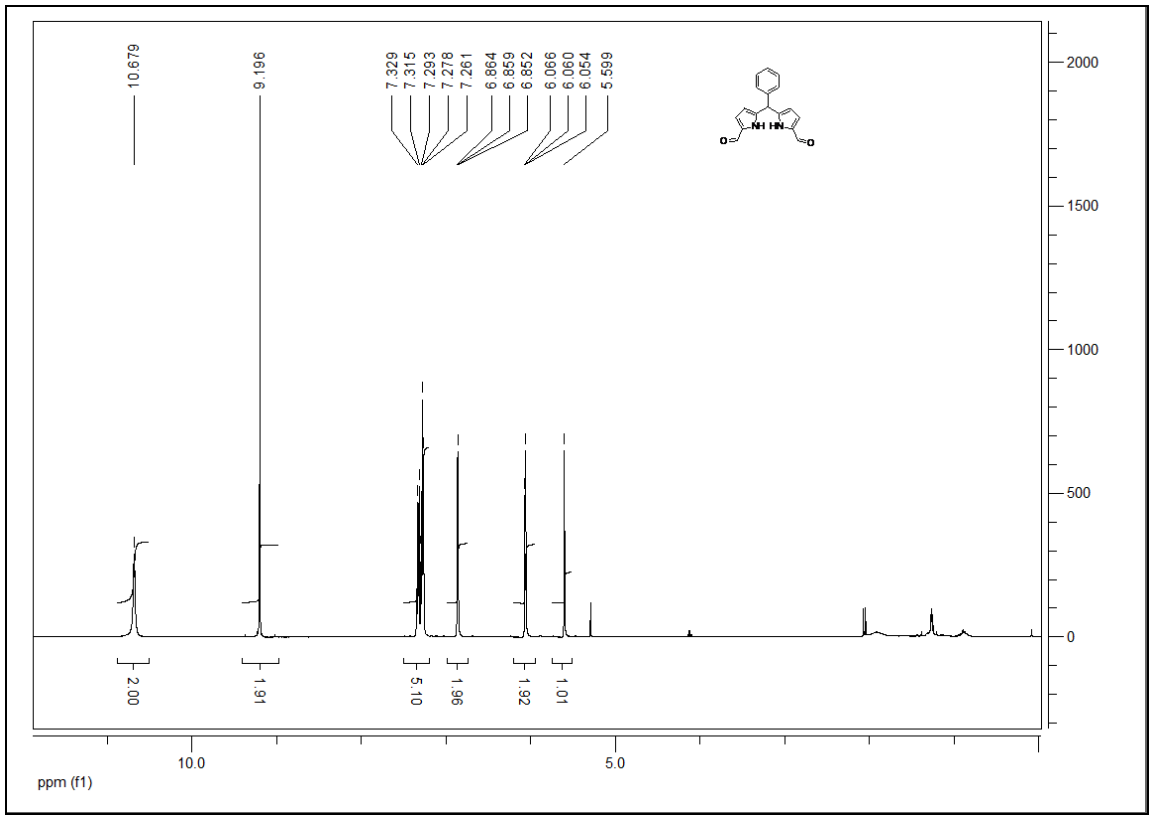
Compound 1a. ^{1}H-NMR spectrum (300MHz, $CDCl_3$)



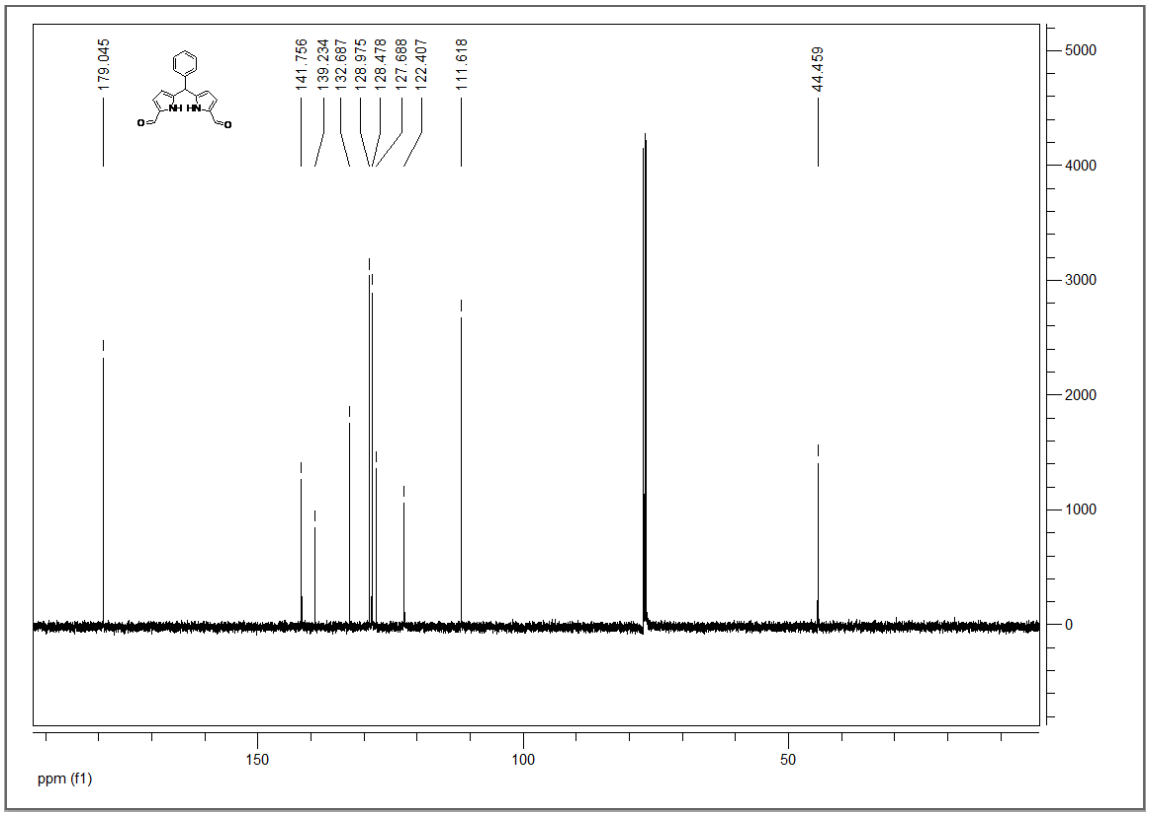
Compound 1a. ^{13}C-NMR spectrum (300MHz, $CDCl_3$)



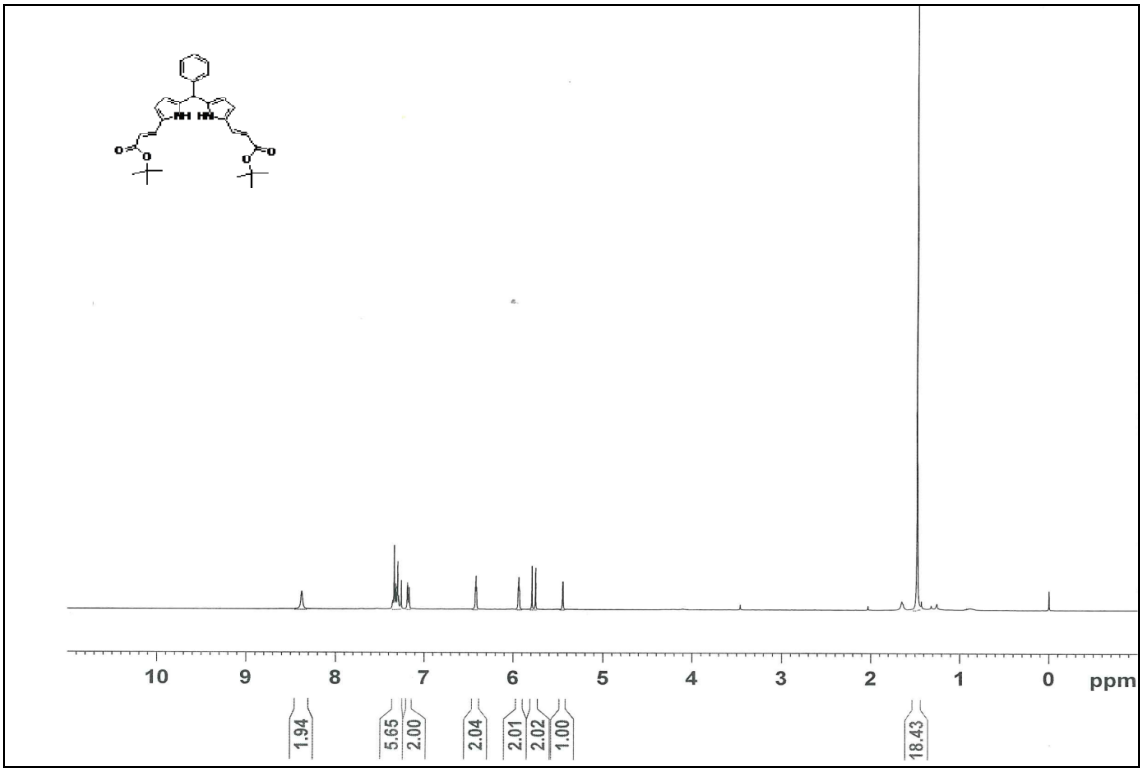
Compound 2a. ^{1}H-NMR spectrum (300MHz,$CDCl_3$)



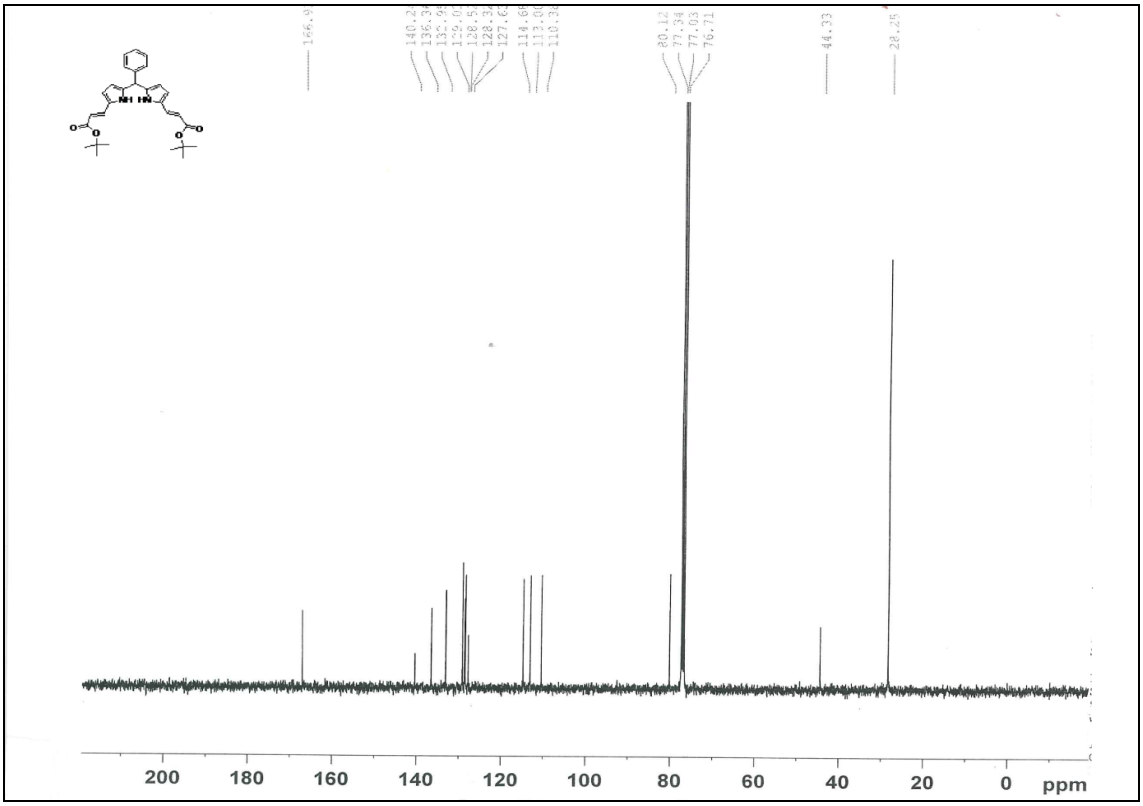
Compound 2a. ^{13}C-NMR spectrum (300MHz, $CDCl_3$)



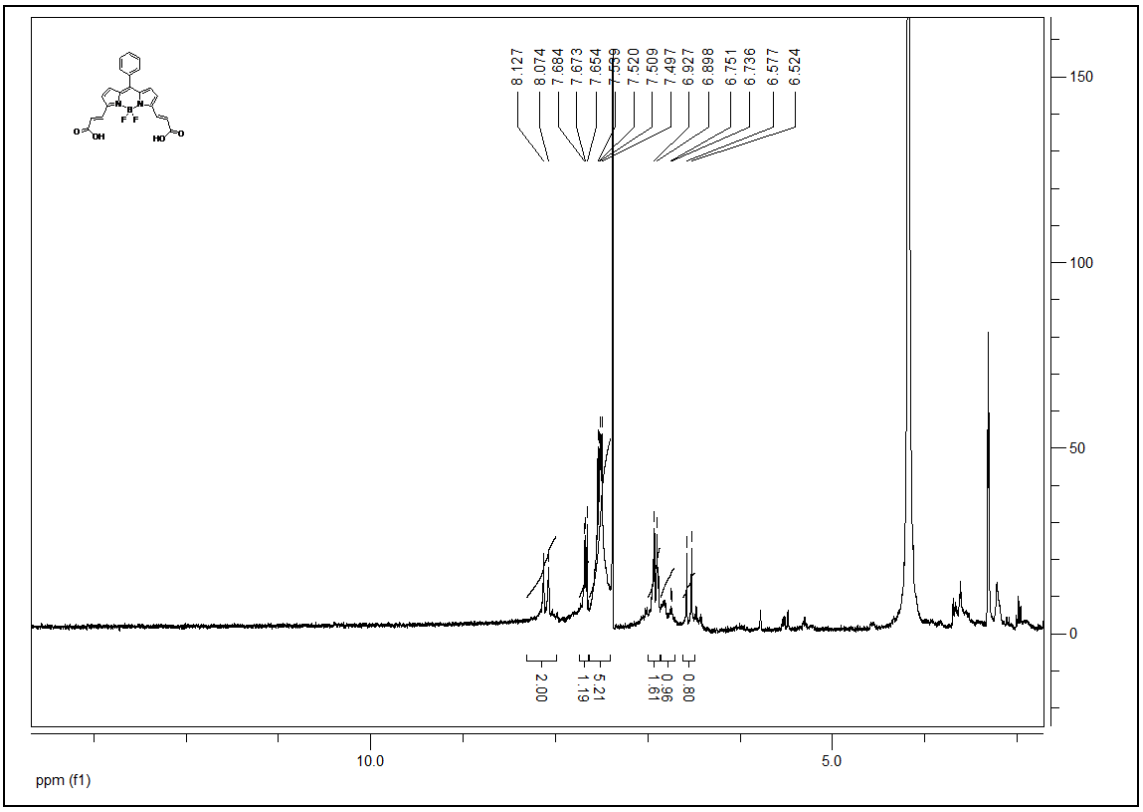
Compound 3a. ^{1}H-NMR spectrum (400 MHz, $CDCl_3$)



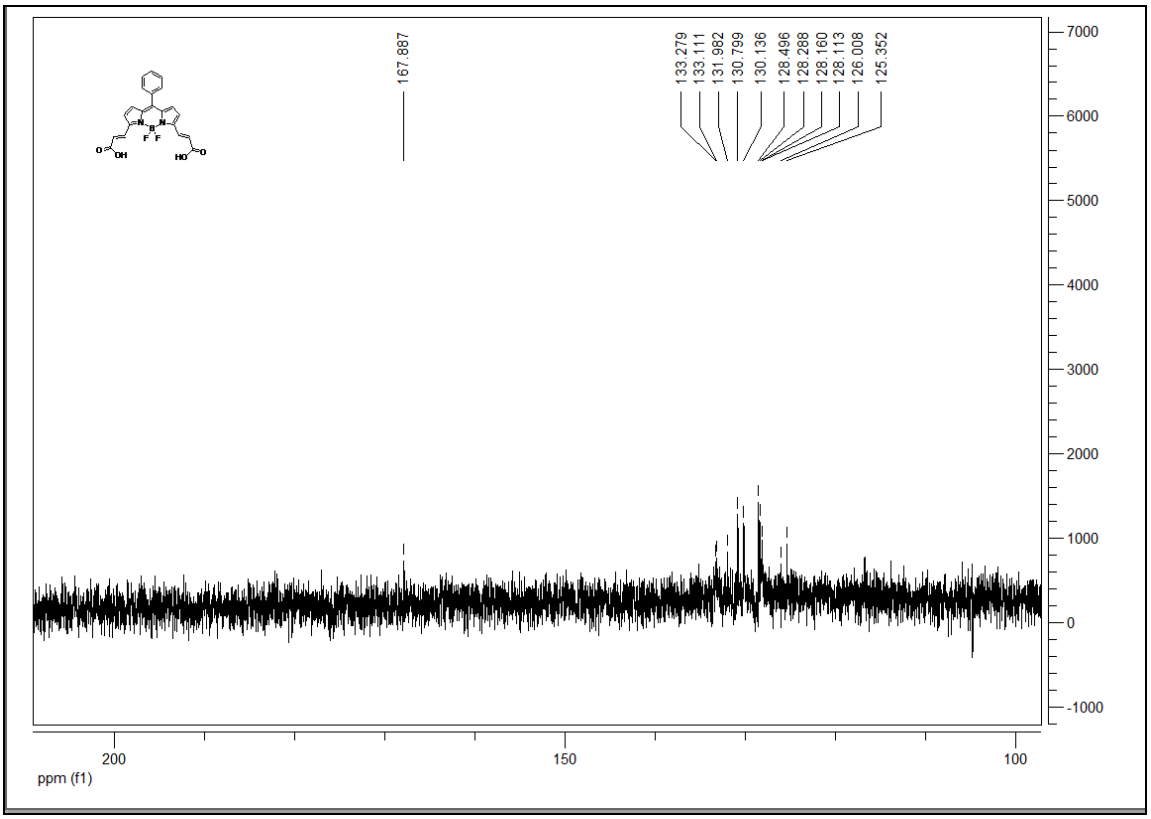
Compound 3a. ^{13}C-NMR spectrum (400 MHz, $CDCl_3$)



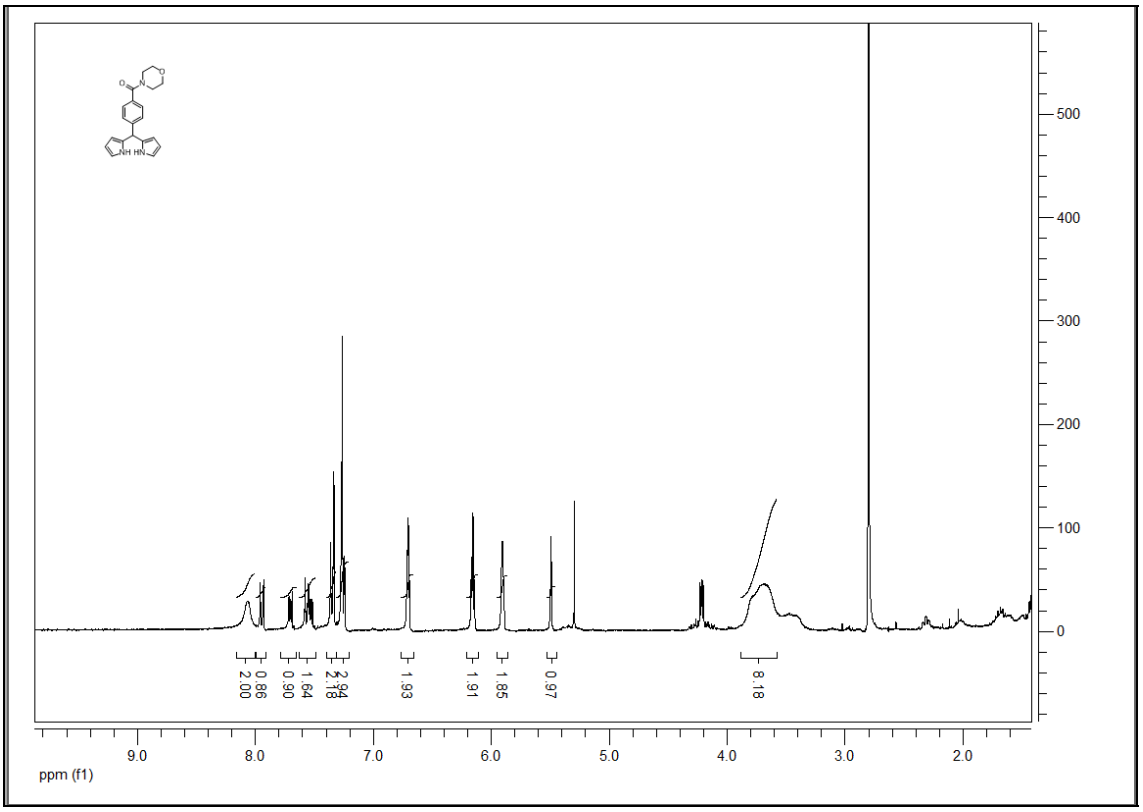
Compound 4a. ^{1}H-NMR spectrum (500 MHz, $CDCl_3$+ CD_3OD)



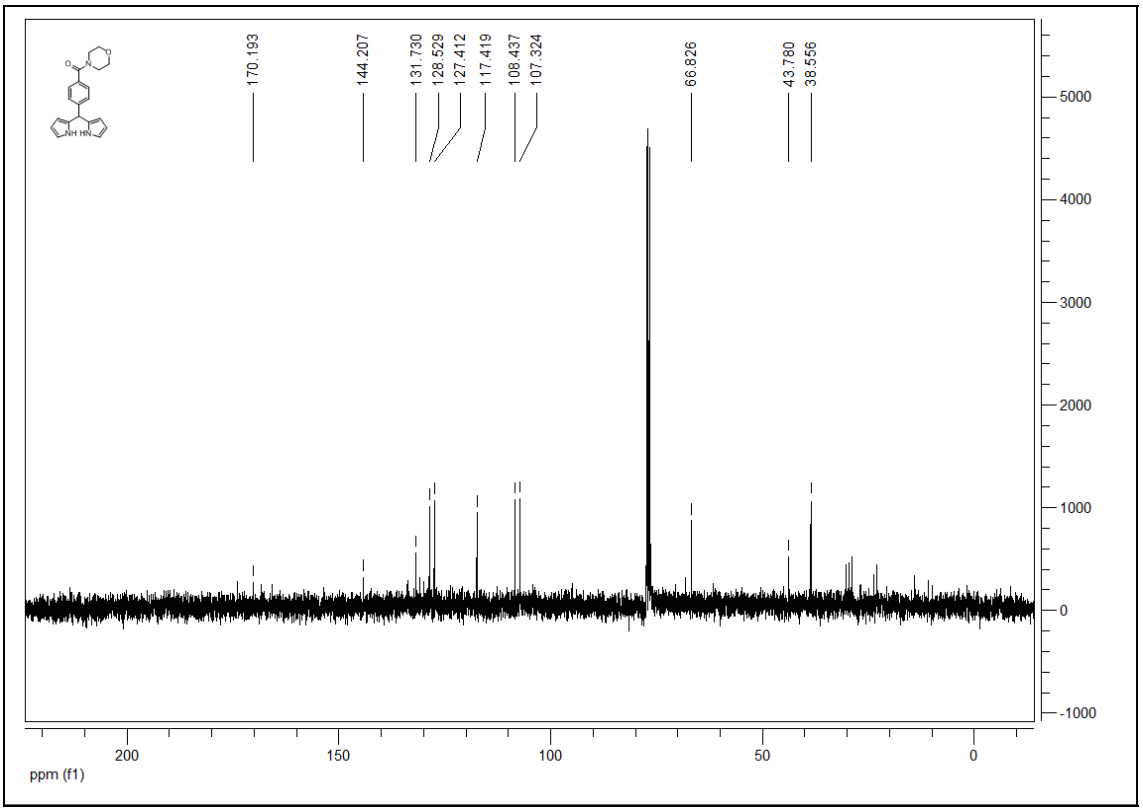
Compound 4a. ^{13}C-NMR spectrum (500 MHz, $CDCl_3$+ CD_3OD)



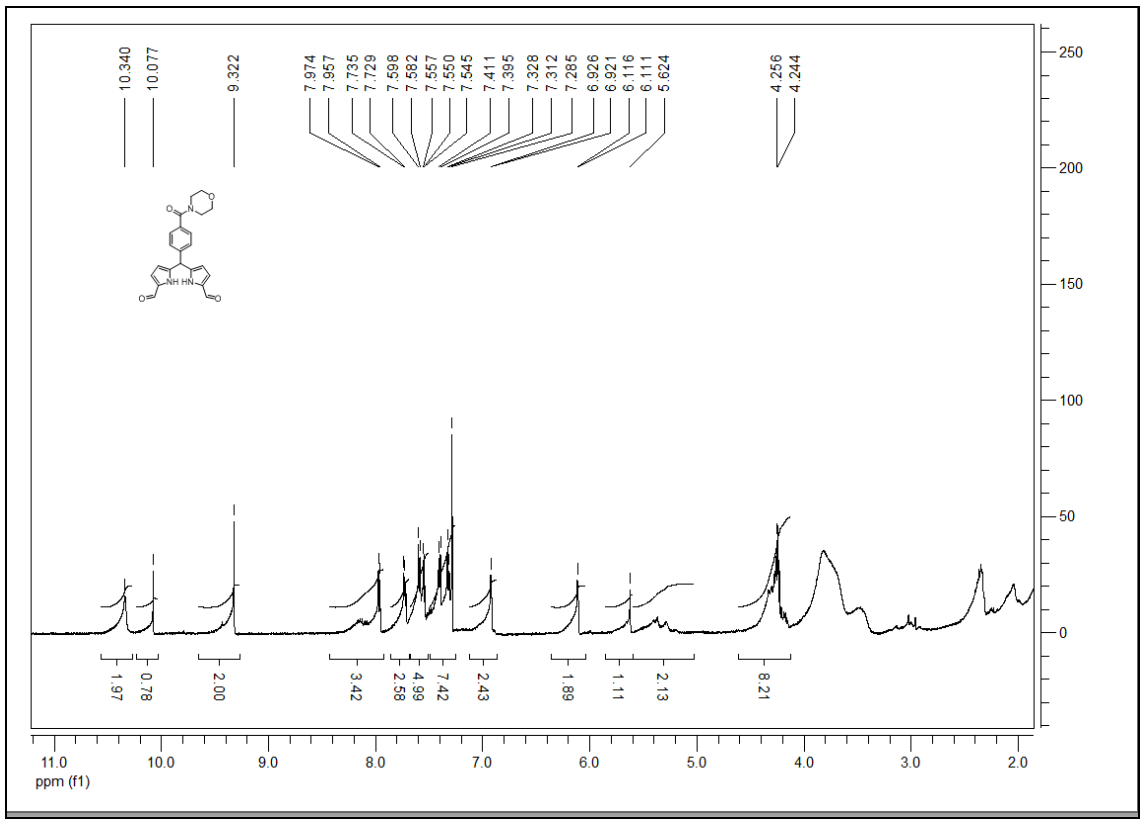
Compound 1b. ^{1}H-NMR spectrum (300MHz, $CDCl_3$)



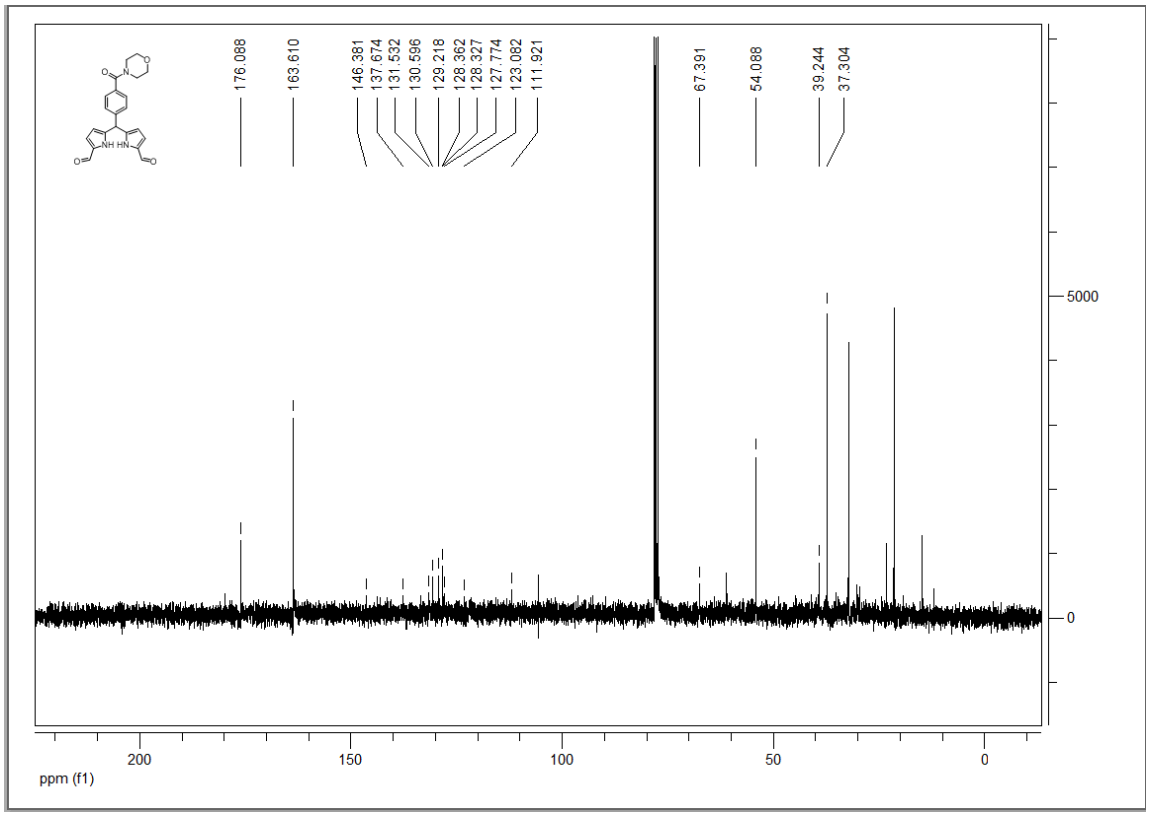
Compound 1b. ^{13}C-NMR spectrum (300MHz, $CDCl_3$)



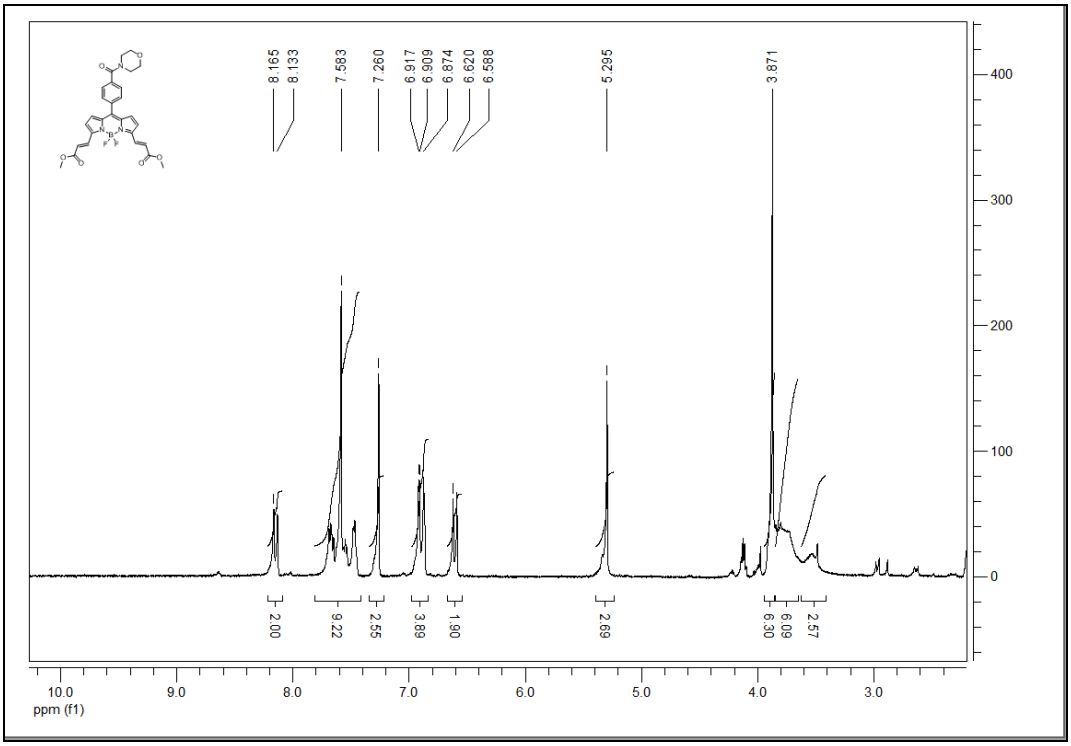
Compound 2b. ^{1}H-NMR spectrum (500MHz, $CDCl_3$)



Compound 2b. ^{13}C-NMR spectrum (500MHz, $CDCl_3$)



Compound 4b. ^{1}H-NMR spectrum (500MHz, $CDCl_3$)



Compound 4b. ^{13}C-NMR spectrum (500MHz, $CDCl_3$)

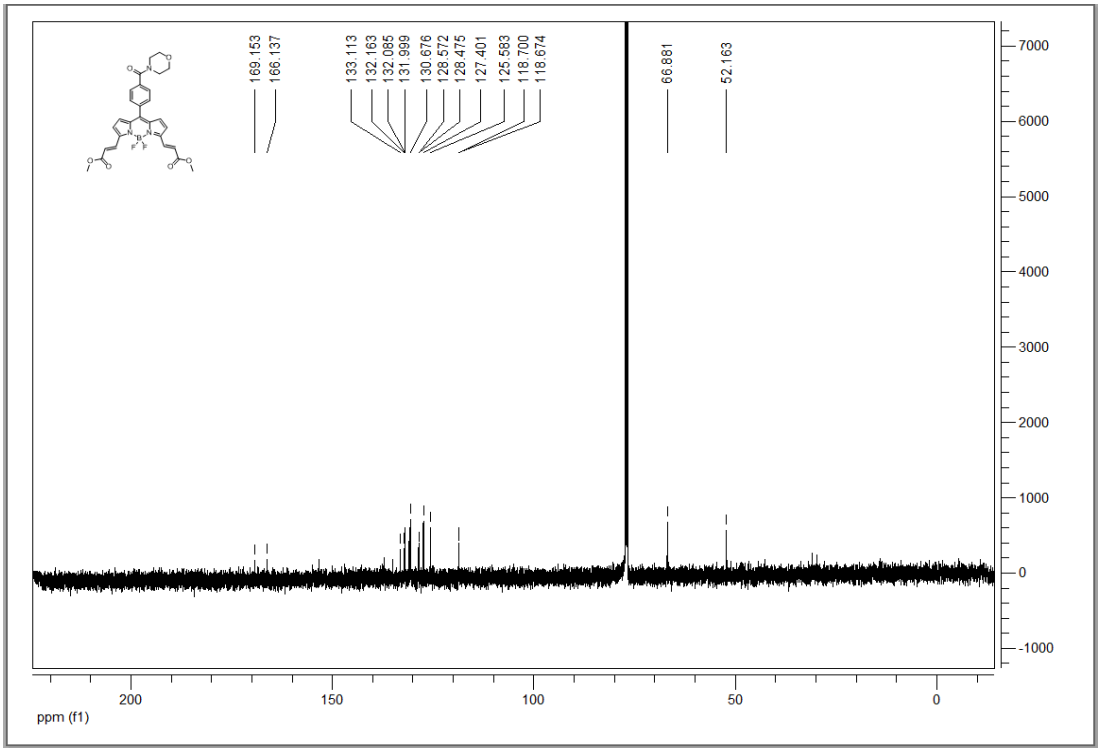


Fig S12. NMR spectrum of listed compounds