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Substituted *meso*-Vinyl-BODIPY as Thiol-Selective Fluorogenic Probes for Sensing Unfolded Proteins in the Endoplasmic Reticulum

Huiying Mu,[a] Koji Miki,*[a] Takuya Kubo,[b] Koji Otsuka[b] and Kouichi Ohe*[a]

a Department of Energy and Hydrocarbon Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan, E-mail: kojimiki@scl.kyoto-u.ac.jp; ohe@scl.kyoto-u.ac.jp

b Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto 615-8510, Japan.

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1. Experimental Section

1.1. Materials and Methods

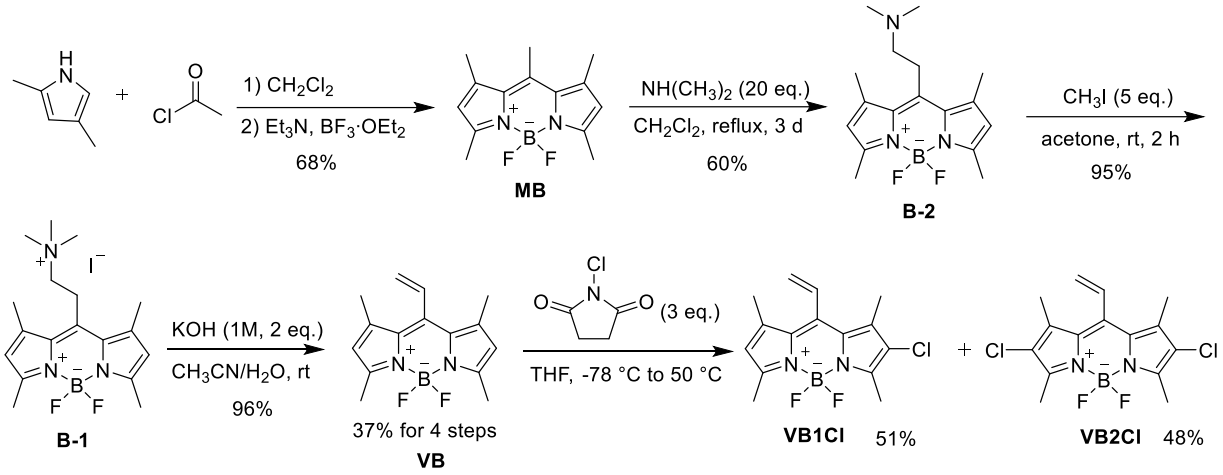
N-chlorosuccinimide, MG132, uranine, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), glycine, leucine, alanine, valine, lysine, and phosphate buffer solution (pH 7.2, 0.1 M) were purchased from Tokyo Chemical Industry (TCI) Co., Ltd. (Japan). Tunicamycin, β-lactoglubulin (LGB), and homocysteine (Hcy) were purchased from Aldrich Chemical Co., New York, USA. Bovine serum albumin, *N*-ethylmaleimide, tyrosine, histidine, serine, threonine, methionine, glutamine, glutamic acid, cysteine, glutathione (GSH, reduced), chloroform ($CHCl_3$), potassium hydroxide (KOH), *N*,*N*-dimethylformamide (DMF), THF, acetone, urea, ethanol (EtOH), and methanol (MeOH) were purchased from nacalai tesque (Japan). Dulbecco's modified Eagle's medium (DMEM) with/without phenol red, dimethylsulfoxide (DMSO), toluene, and acetonitrile (CH_3CN) were purchased from Wako Pure Chemicals Inc. (Japan). Dichloromethane (CH_2Cl_2) was purchased from Kishida Chemical Co., Ltd. (Japan). LysoTracker™ Deep Red, ER-Tracker™ Red, MitoTracker™ Deep Red were purchased from Thermo Fisher Scientific, Inc. USA. Silica gel (SiO_2, 230–400 mesh) for column chromatography was purchased from Silicycle (Canada). Buffered aqueous solutions (pH 2.5–5.8) were prepared by dissolving citric acid and sodium dihydrogenphosphate (NaH_2PO_4) in water (MilliQ). Buffered aqueous solutions (pH ~7.4) were prepared by dissolving NaH_2PO_4 and disodium hydrogenphosphate (Na_2HPO_4) in water (MilliQ). Buffered aqueous solutions (pH 9.2–11.5) were prepared by dissolving sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3) in water (MilliQ). All buffered aqueous solutions were stored in refrigerator and used within one week.

UV-vis absorption spectra of dyes were measured by UV-vis-NIR spectrophotometer (UH5300, Hitachi High-Technologies Co., Japan). The sample solutions were prepared by mixing a solution of dyes (5.0×10^{-6} M) in DMSO (5 μL, 3×10^{-3} M) with different concentration of biothiols in MeCN/PBS (pH 7.2, v/v=1/1, 3 mL) or deoxygenated buffered aqueous solution (pH 7.2, 3 mL) for 1 h incubation.

Emission spectra of dyes were measured by fluorescence spectrophotometer (RF-6000, Shimadzu Co., Japan). The sample solutions were prepared in the same manner shown in the method of UV-vis absorption measurement.

High resolution mass spectra were measured by EXACTIVE (ESI, Thermo Fisher Scientific Inc., USA). Confocal microscopy was performed on LSM 710 (Carl Zeiss, Germany). Circular dichroism (CD) spectra were measured by circular dichroism spectrometer (JASCO J-820, JASCO Co., Japan).

1.2. Synthesis of Vinyl-Bodipy derivatives VB, VB1Cl, and VB2Cl



Scheme S1. Synthesis of **VB**, **VB1Cl**, and **VB2Cl**.

Synthesis of **VB**. Firstly, compound **B-1** was prepared by following the procedure in reported work.[1] The solution of compound **B-1** (70 mg, 0.15 mmol) in 3 mL mixture solvent (CH_3CN/H_2O, v/v = 3/1) was placed in a round-bottomed flask and stirred at 0 °C. Then the aqueous solution of KOH (1 M, 2 eq) was added dropwise. After that the contents were stirred at room temperature and the reaction was monitored by TLC plate (CH_2Cl_2/MeOH=100/1). The solid was precipitated with time passing, the product **VB** was obtained by filtration as orange-red solid (40 mg, 0.15 mmol, 96%).

Synthesis of **VB1Cl** and **VB2Cl**. To a stirred solution of compound **VB** (22 mg, 78 μmol) in dry THF (4 mL) at –78 °C under N_2 atmosphere was added dropwise a solution of *N*-chlorosuccinimide (31 mg, 0.24 mmol, 3 eq.) in THF (2 mL) through a syringe pump over 30 min. The resulting mixture was slowly heated to 50 °C until starting material totally consumed and the ending point was confirmed by TLC (hexane/CH_2Cl_2 = 1/1). After solvents were removed under reduced pressure, the residue was diluted into CH_2Cl_2 and washed with brine, dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography on silica gel using hexane/MeOH as the mobile phase to afford **VB1Cl** (12 mg, 39 μmol, 51%) and **VB2Cl** (13 mg, 38 μmol, 48%).

VB1Cl ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.72 (q, *J* = 12 Hz, 1H), 6.01 (s, 1H), 5.67 (d, *J* = 12 Hz, 1H), 5.53 (d, *J* = 12 Hz, 1H), 2.47 (s, 6H), 2.18 (s, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 157.7, 149.9, 144.3, 141.0, 135.8, 131.2, 129.2, 126.7, 124.6, 122.3, 121.8, 17.4, 14.8, 12.5. TOF MS: m/z calcd for $C_{15}H_{15}BClF_2N_2^-$ $[M–H]^-$ 307.0990, found: 307.0991.

VB2Cl ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.81 (q, *J* = 12 Hz, 1H), 5.78 (d, *J* = 12 Hz, 1H), 5.61 (d, *J* = 12 Hz, 1H), 2.55 (s, 6H), 2.24 (s, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 152.0, 141.1, 137.1, 130.7, 128.9, 124.9, 122.5, 14.7, 12.4. TOF MS: m/z calcd for $C_{15}H_{14}BCl_2F_2N_2^-$ $[M–H]^-$ 341.0601, found: 341.0598.

1.3. X-ray crystallographic data

Single crystals of **VB1Cl** were obtained from recrystallization in CH_2Cl_2/hexane mixture at 25 °C. Intensity data were collected on a RIGAKU Saturn 724 HG CCD system with VariMax Mo Optic using MoKα radiation. Crystal data are summarized in Table S1. The structures were solved by a direct method and refined by a full-matrix least square method on F^2 for all reflections. All hydrogen atoms were placed at positions with high electron density, while all other atoms were refined anisotropically. The occupancies of Cl1 and Cl2 atoms are 0.80 and 0.20, respectively. The electron density nearby Cl1 atom which is suitable for a hydrogen atom was not found due to large electron density of Cl1 atom. Since the electron density of Cl2 atom (occupancy: 0.20) is still large, the position of the hydrogen atom H13 on BODIPY core was not properly assigned. Therefore, the bond length (0.66(2)) between C8 and H13 was shorter than normal C–H bond (Alert A). The occupancy of H13 atom was set to 1.0 and refined. Supplementary crystallographic data were deposited at the Cambridge Crystallographic Data Centre (CCDC) under the number CCDC-2034710 and can be obtained free of charge from web site: www.ccdc.cam.ac.uk/data_request/cif. The molecular structure was visualized by Mercury provided from CCDC.

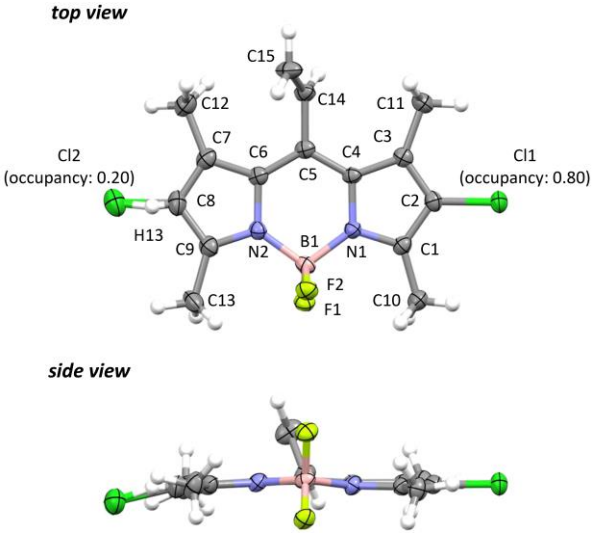


Figure S1. Thermal ellipsoid drawing (50% probability level) of **VB1Cl**. ∠C4–C5–C14–C15 = 111.11°.

Table S1. Crystallographic data for **VB1Cl**.

empirical formula	$C_{15}H_{16}N_2BClF_2$	D_{calc} (g·cm^{-3})	1.417
formula weight	308.56	μ (cm^{-1})	2.788
temperature (°C)	–130.0	2θ(°)	55.0
crystal color	orange	reflections collected	5879
crystal size (mm)	0.30×0.10×0.05	independent reflections	3162
crystal system	triclinic	R_{int}	0.0370
space group	P-1 (#2)	No. of variables	263
a (Å)	8.337(8)	reflection/parameter ratio	12.02
b (Å)	8.954(9)	goodness of fit	1.000
c (Å)	10.867(11)	R_1 [$I>2\sigma(I)$]	0.0410
α (˚)	109.950(10)	R_1 (all data)	0.0645

β (˚)	102.666(11)	wR_2 (all data)	0.0785
γ (˚)	98.075(6)	maximum peak in final diff. map (e·Å^3)	0.38
V (Å^3)	723.2(12)	minimum peak in final diff. map (e·Å^3)	–0.31
Z	2		

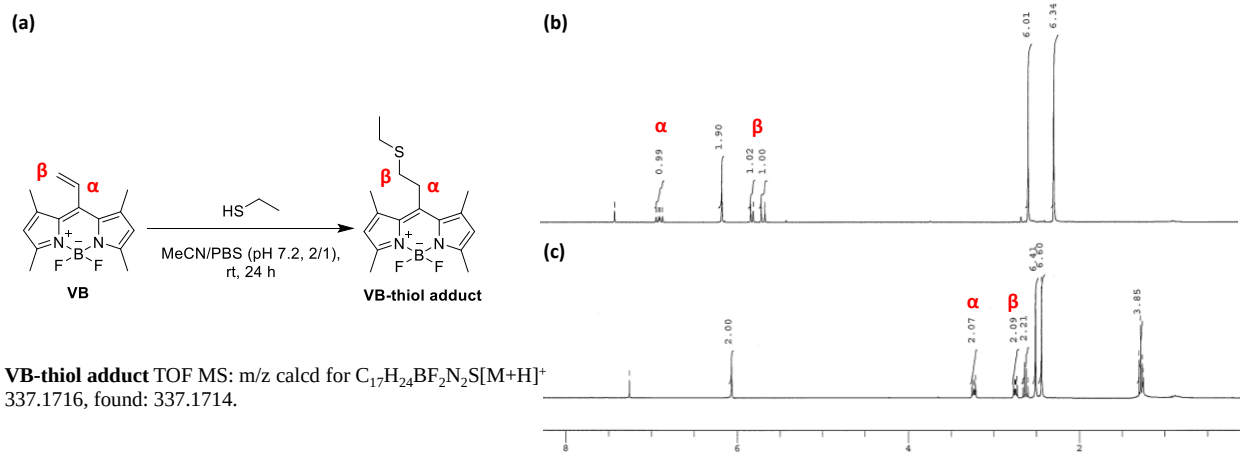
1.4. Evaluation of photophysical properties of probes VBs

The relative fluorescence quantum yields of probes and corresponding product were determined by fluorescence spectrophotometer (RF-6000, Shimadzu Co., Japan) according to their absorbance intensity. We chose uranine as standard, which has a fluorescence quantum yield of 0.97 in ethanol.[2] The cLogP was estimated by ChemDraw software (PerkinElmer Informatics, Inc., USA).

Table S2. Photophysical properties of compounds determined in EtOH at 25 °C.

compound	λ_{ab} (nm)	λ_{em} (nm)	ε [M^{-1}cm^{-1}]	Φ_{FL}	cLogP
VB	499	526	75000	0.0039	4.03
VB1Cl	512	525	53000	0.0029	4.75
VB1Cl+GSH	512	526	53000	0.9805	2.34
VB2Cl	528	543	55000	0.0055	5.47

1.5. ^{1}H-NMR spectra of probe VB and VB-thiol adduct



VB-thiol adduct TOF MS: m/z calcd for $C_{17}H_{24}BF_2N_2S[M+H]^+$ 337.1716, found: 337.1714.

Figure S2. (a) Addition reaction of **VB** with ethanethiol. ^{1}H NMR spectra of (b) **VB** and (c) **VB-thiol adduct** in $CDCl_3$.

1.6. Reactivity of probe VBs towards Cysteine (Cys)

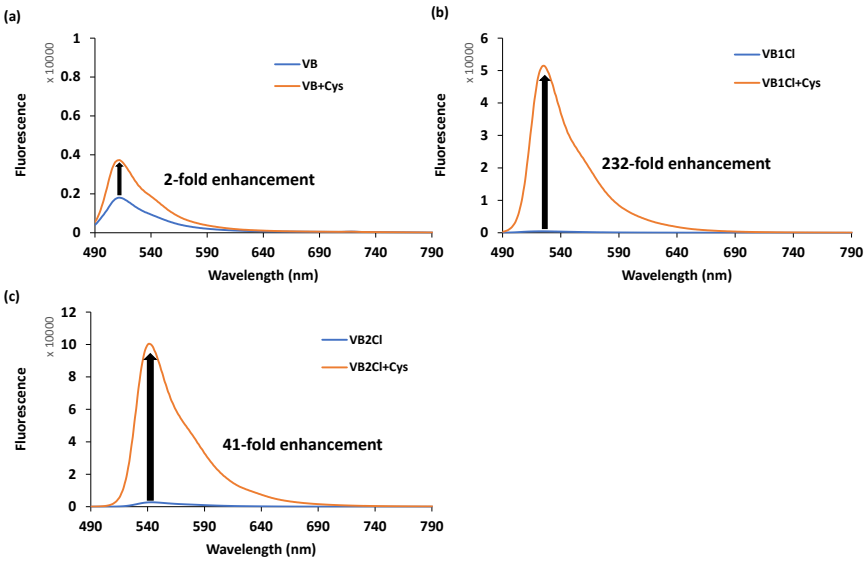


Figure S3. Fluorescence spectra of (a) **VB**, (b) **VB1Cl**, and (c) **VB2Cl** (5 μM) in absence and presence of Cys (1 mM) in PBS/MeCN (1/1) solution after 1 h incubation. λ_{ex} = 480 nm.

1.7. Titration assays for VB1Cl with biothiols

The sample solutions were prepared by mixing dye **VB1Cl** (5 μM) with different concentration of biothiols (Cys, GSH and Hcy) in PBS/MeCN (pH 7.2, 0.1 M, v/v=1/1) solution and incubated at room temperature for 1 h. Excitation wavelength: 480 nm.The UV-vis absorption and fluorescence spectra were summarized as below. The reaction adducts of each solution were confirmed by HR-MS spectra. **VB1Cl-Hcy**: TOF MS: m/z calcd for $C_{19}H_{25}BClF_2N_3O_2S^+$ $[M+Na]^+$ 466.1309, found: 466.1485. **VB1Cl-Cys**: TOF MS: m/z calcd for $C_{18}H_{23}BClF_2N_3O_2SNa^+$ $[M+Na]^+$ 452.1153, found: 452.1155. **VB1Cl-GSH**: TOF MS: m/z calcd for $C_{25}H_{33}BClF_2N_5O_6S^-$ $[M–H]^-$ 614.1828, found: 614.1828.

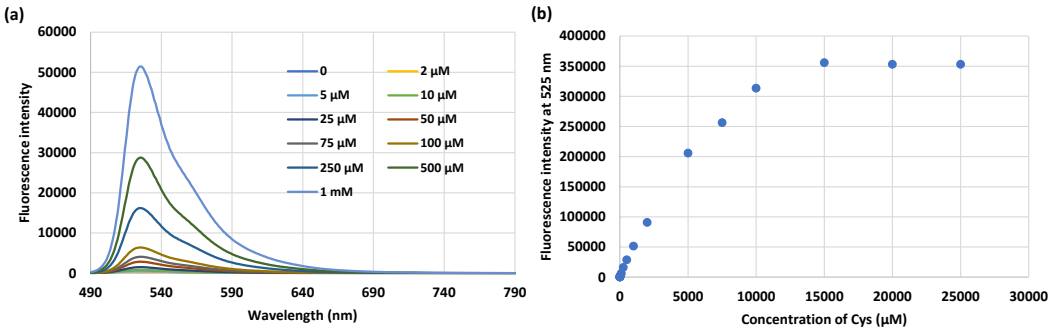


Figure S4. (a) Fluorescence spectra of **VB1Cl** (5 μM) in absence or presence of Cysteine (Cys, 0–1 mM) in MeCN/PBS (1/1) after 1 h incubation. (b) fluorescence intensity of **VB1Cl** at 525 nm vs Cys (0–25 mM).

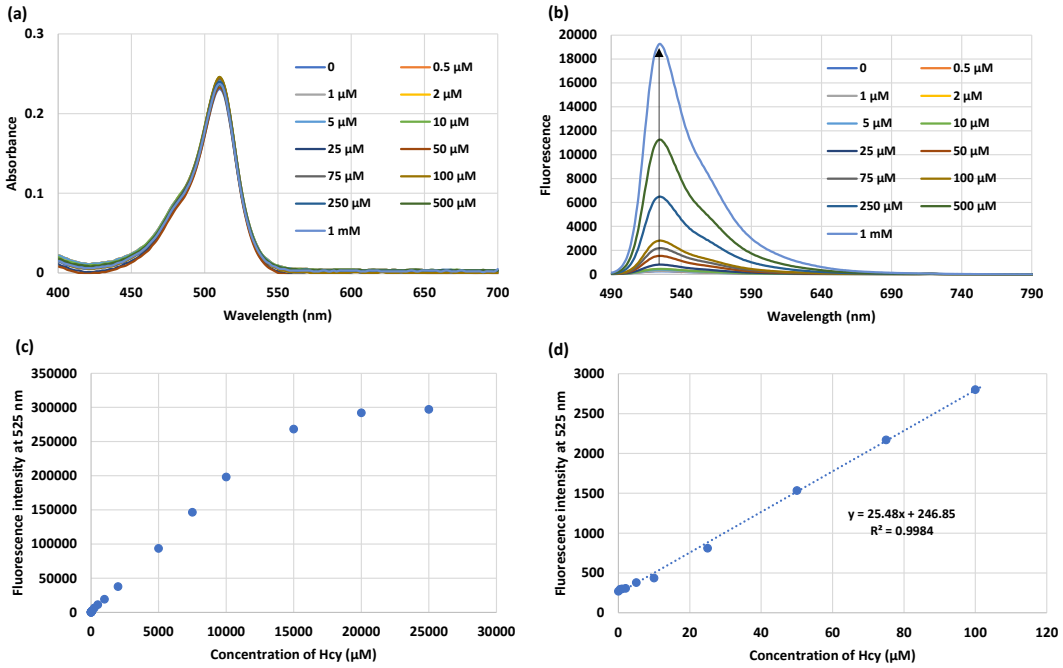


Figure S5. (a) UV-vis absorbance and (b) fluorescence spectra of **VB1Cl** (5 μM) in absence or presence of Hcy (0–1 mM) in MeCN/PBS (1/1) after 1 h incubation. (c) fluorescence intensity of **VB1Cl** at 525 nm vs Hcy (0–25 mM). (d) Calibration curve of fluorescence intensity at 525 nm vs Hcy (0–100 μM).

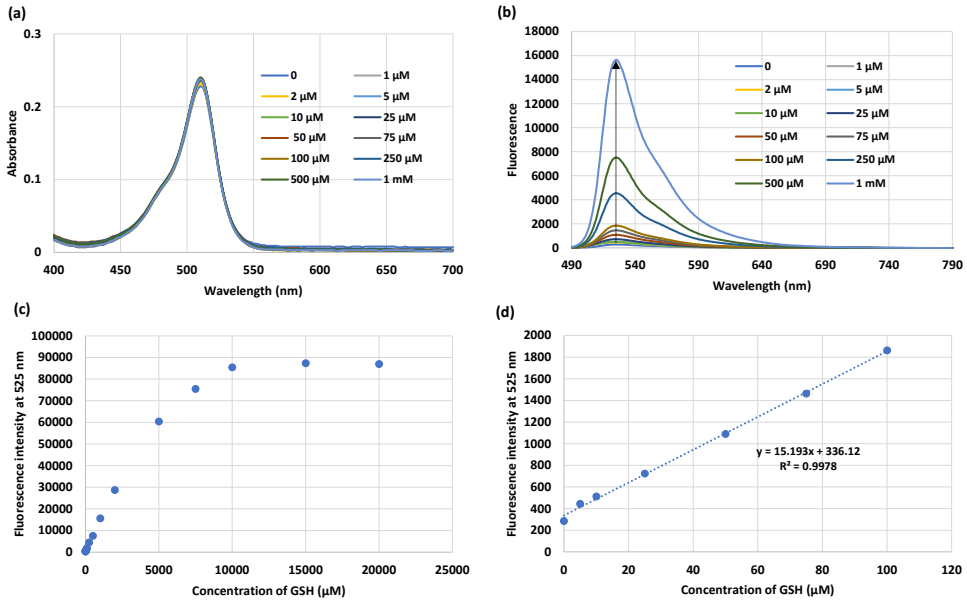


Figure S6. (a) UV-vis absorbance and fluorescence spectra of **VB1Cl** (5 μM) in absence or presence of GSH (0–1 mM) in MeCN/PBS (1/1) after 1 h incubation. (c) fluorescence intensity of **VB1Cl** at 525 nm vs Hcy (0–25 mM). (d) Calibration curve of fluorescence intensity at 525 nm vs GSH (0–100 μM).

1.8. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration curve of **VB1Cl** in the presence of Cys, Hcy, GSH (0–100 μM, Figures 2d, S5d, and S6d), and unfolded protein (LGB, 0–250 μM, Figure 3c). The fluorescence intensity of **VB1Cl** was measured by three times and the standard deviation of blank measurement was achieved. The detection limit was calculated by using detection limit was calculated with the following equation:

Detection limit = 3σ/k;

Where σ is the standard deviation of the blank measurement, k is the slope between the fluorescence ratios versus thiols' concentration.

The detection limit of **VB1Cl** towards Cys, Hcy, GSH and unfolded protein LGB was calculated as 0.97, 1.86, 3.14 μM,and 0.56 mg/L respectively.

1.9. pH-dependent assay

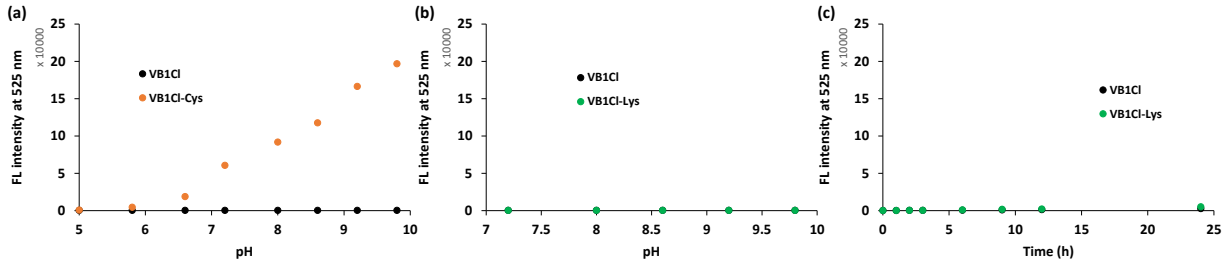


Figure S7. pH-dependent fluorescence intensity at 525 nm of **VB1Cl** (5 μM) towards (a) Cys and (b) Lys (1 mM) in MeCN/PBS (1/1, 0.1 M) solution. Incubation time: 1 h. (c) Time-dependent fluorescence of **VB1Cl** (5 μM) in absence and presence of Lys (1 mM) in MeCN/PBS (1/1, 0.1 M, pH 9.8) solution. λ_{ex} = 480 nm.

1.10. Time-dependent assays for VB1Cl and VB1Cl-GSH adduct after HPLC purification

HPLC analytical experiment was performed to isolate the VB1Cl-GSH adduct by instrument equipped with HPLC column oven, UV detector (GL-7450, GL Sciences Inc., Japan), and YMC-Triart Phenyl column (250 × 4.6 mm, 5 μm) (YMC Co., Ltd, Japan). The mobile phase consisted of water (phase A) and acetonitrile (phase B). The gradient program was set as follows: 0–10 min, 100% B; 10–35 min, 0% B. The flow rate was 1.0 mL/min with a pump (GL-7410, GL Sciences Inc., Japan) with UV detector at 250 nm.

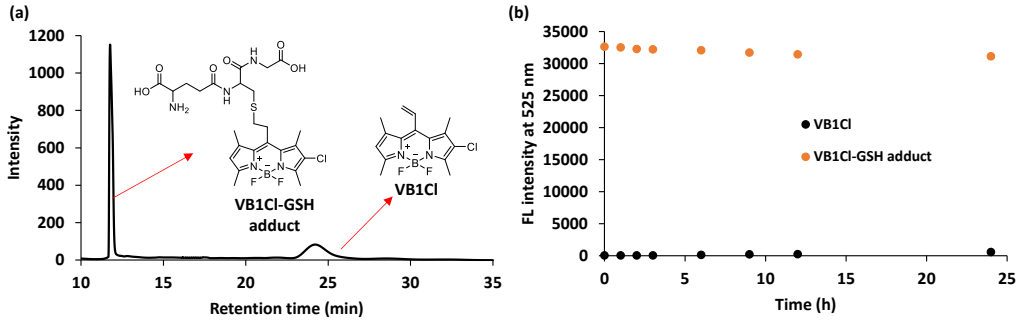
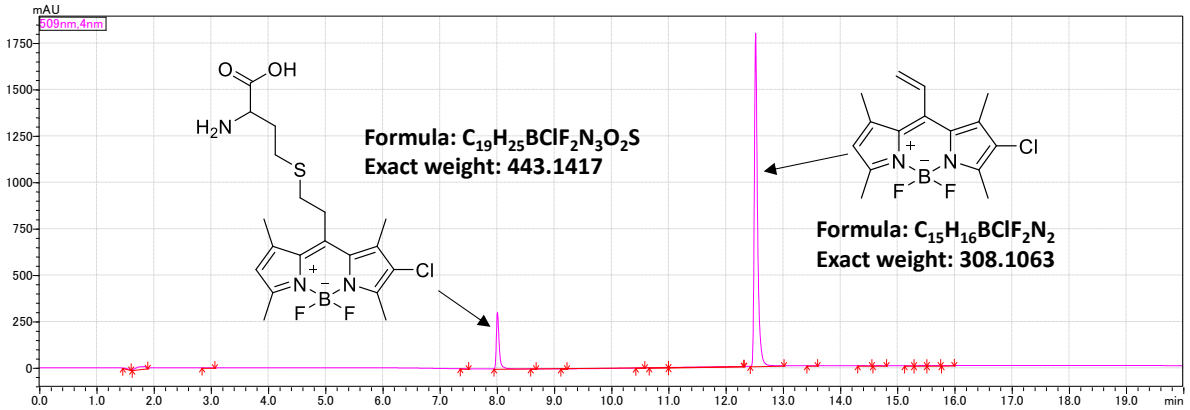


Figure S8. (a) UV–vis trace of HPLC chromatogram for isolation of **VB1Cl** and **VB1Cl-GSH** adduct. The retention time for **VB1Cl**, **VB1Cl-GSH adduct** was assigned as 24.2 min and 12.8 min, respectively. (b) Time-dependent fluorescence at 525 nm of **VB1Cl** and VB1Cl-GSH adduct (1 μM) in MeCN/PBS (1/1, 0.1 M, pH = 9.8) solution. λ_{ex} = 480 nm.

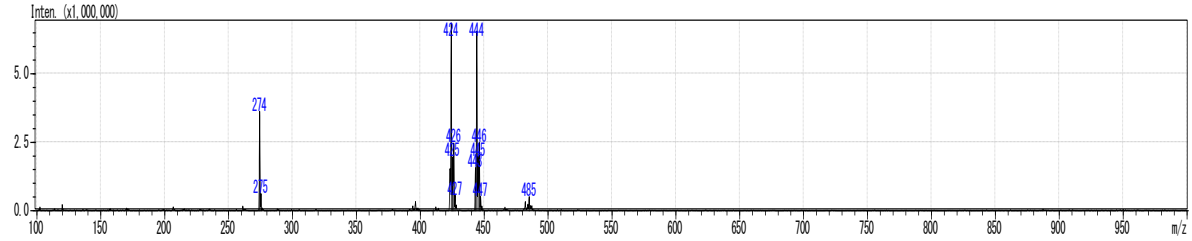
1.11. LC-MS/MS analysis of VB1Cl toward Hcy, Cys, and GSH

Liquid chromatography-tandem mass spectrometer (LC-MS/MS) was carried out on an Nexera X2 UHPLC liquid chromatograph system (LC-30AD, Shimadzu Industrial System Co., Ltd. Japan) with an Mightysil RP II column (150 * 4.6 mm) and PDA detector (SPD M30A). The mobile phase consisted of 0.1% formic acid aqueous solution (phase A) and acetonitrile (phase B). The gradient program was set as follows: 0–3 min, 30% B; 3–10 min, 30–100% B; 10–20 min, 100% B; 20–27 min, 30% B. The flow rate was 1.0 mL/min with a pump (LC-30AD, Shimadzu Industrial System Co., Ltd. Japan). Detection was performed on a triple quadrupole mass spectrometer (LCMS-8030, Shimadzu Industrial System Co., Ltd. Japan) with an electrospray ionization (ESI) source. The parameters of the source were set under a N_2 generator (AT 10NP5NSC). The MS was operated in the positive or negative ionization mode with the data acquisition mode of selective ion monitoring (SIM). The sample solutions were prepared by mixing dye **VB1Cl** (100 μM) with different concentration of biothiols (Cys, GSH and Hcy, 100 μM) in PBS/MeCN (pH 7.2, 0.1 M, v/v=1/1) solution.

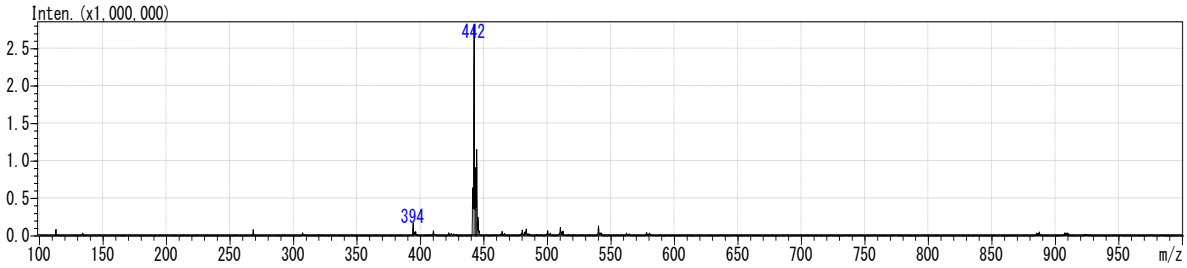
(a)



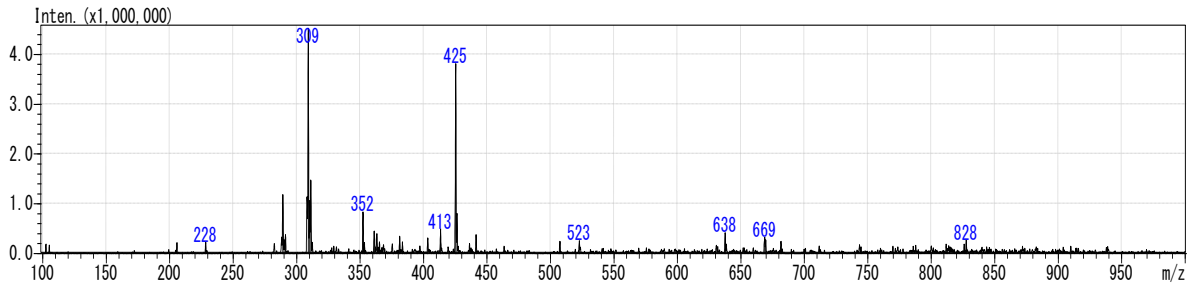
Positive mode, retention time: [8.082->8.170]-[7.993<->9.273]



Negative mode, Retention time: [8.104->8.192]-[8.015<->9.295]



Positive mode, retention time: [12.539->12.627]-[12.451<->13.289]



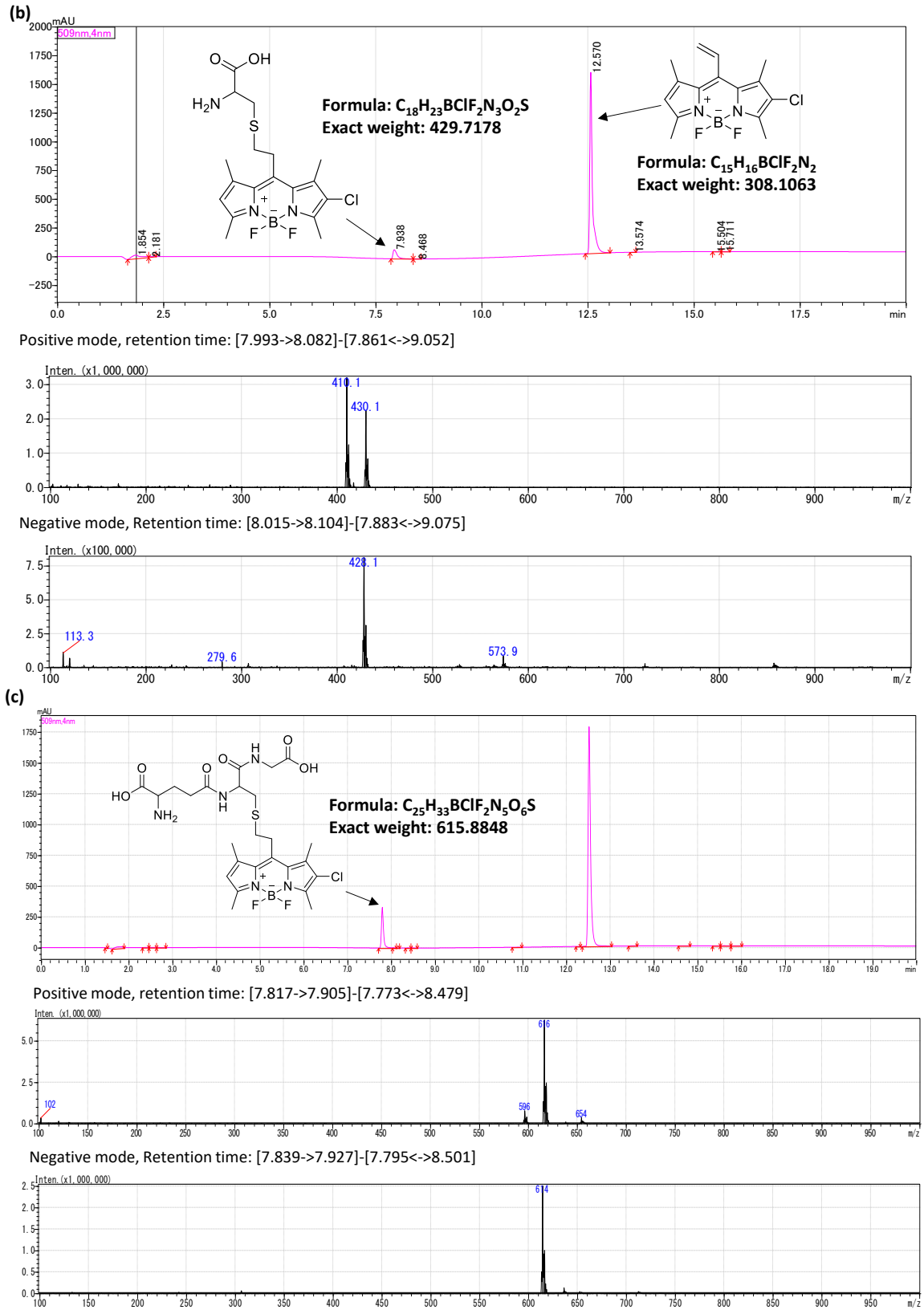


Figure S9. HPLC-MS analysis of reaction adduct **VB1Cl** with (a) Hcy, (b) Cys, and (c) GSH.

1.12. Theoretical calculation

The density function theory (DFT) calculations were performed for **VB1Cl** and adduct **VB1Cl+Cys** at the B3LYP/6-31G (d) level (for the ground state) and CAM-B3LYP/6-31G(d) level (for the excited state) by using Gaussian 16 package.[3] Cartesian coordinates of all compounds are shown at the end of the SI.

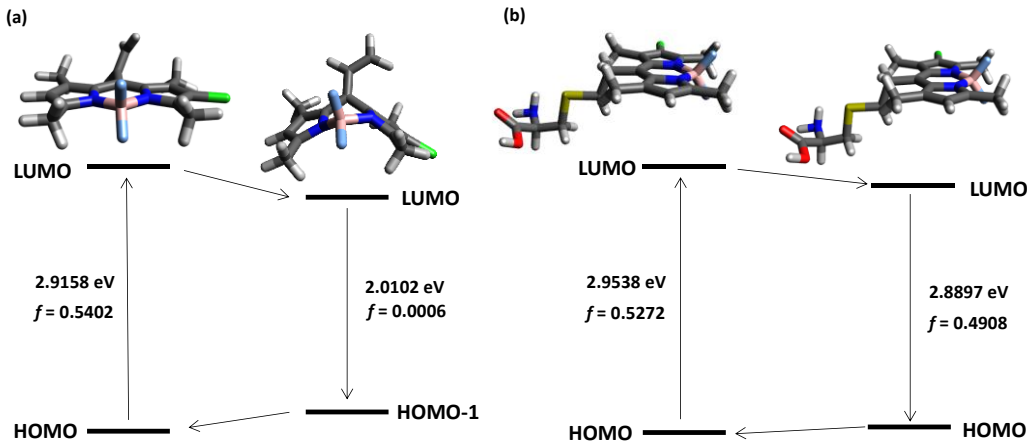


Figure S10. Calculation energy diagrams of (a) **VB1Cl** and (b) **VB1Cl+Cys** adduct at B3LYP/6-31G(d) level. The optimized geometries of Ground state (left) and excited state (right) are shown.

1.13. CD and absorbance spectra of LGB

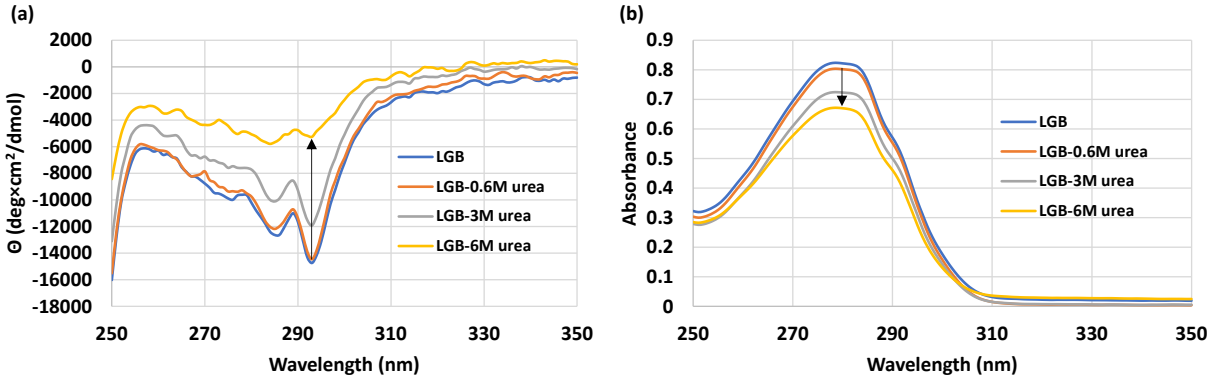
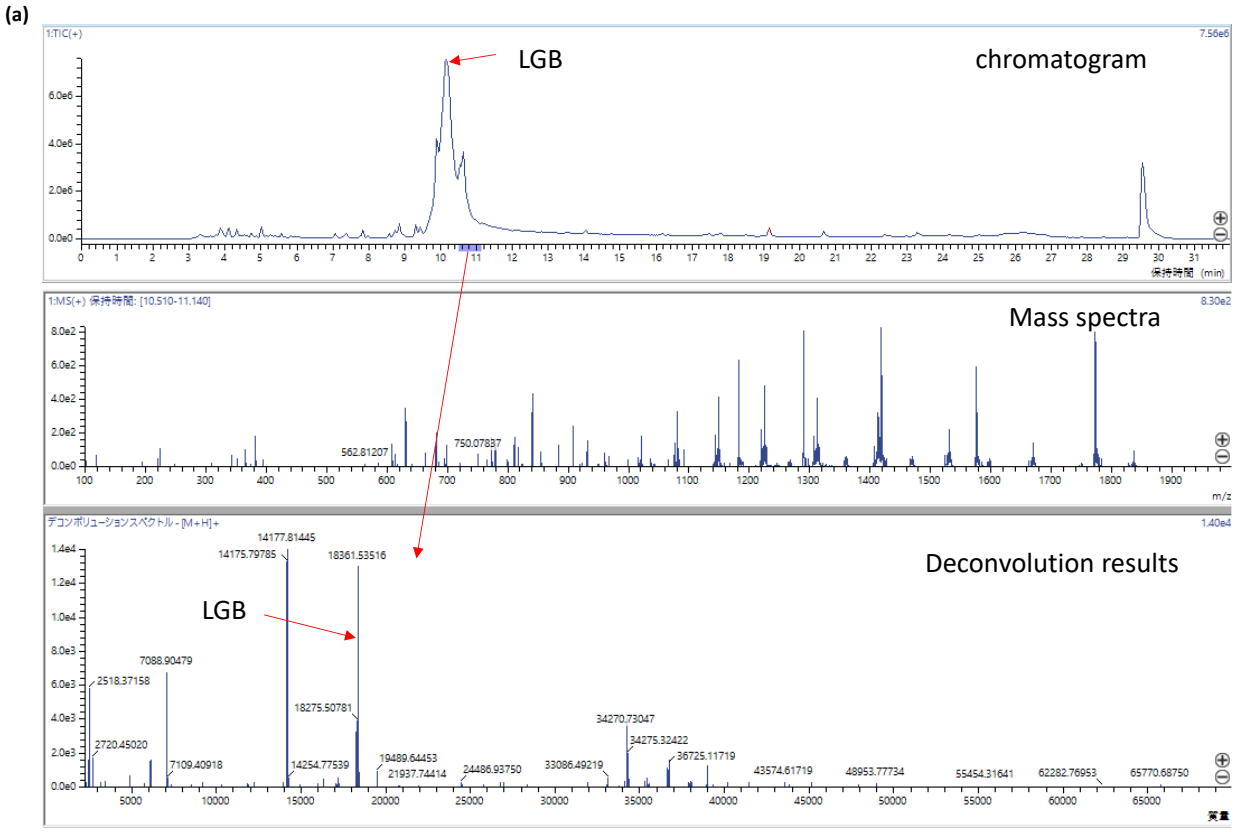


Figure S11. (a) Typical near-UV circular dichroism (CD) and (b) absorbance spectra of β-lactoglobulin (250 μM) treated with different concentration of urea (0–6 M).

1.14. LC-Mass analysis of protein labeling

Protein mass spectrometry was obtained on Quadrupole Time of Flight Liquid Chromatography Mass Spectrometer (Q-TOF LCMS-9030, Shimadzu Industrial System Co., Ltd., Japan) with an Triart Bio C4 column (150 * 2.1 mm I.D., 3μm, 30 nm, YMC Co., Ltd., JAPAN) at 40 °C. The mobile phase consisted

of 10 mM NH_4HCO_3 aqueous solution (pH 7.8) (phase A) and acetonitrile (phase B). The gradient program was set as follows: 0–1 min, 20% B; 25–27 min, 100% B; 29–32 min, 20% B. The flow rate was 0.3 mL/min with a pump (LC-40B, Shimadzu Industrial System Co., Ltd., Japan). Injection volume was 10 μL. Detection was performed on a triple quadrupole mass spectrometer (LCMS-9030, Shimadzu Industrial System Co., Ltd., Japan) with an electrospray ionization (ESI) source. Mass range was set at 100-3000 m/z. Workstation of measurement was carried out in LabSolutions LCMS Ver. 5.99 SP2. The sample solutions were prepared by mixing unfolded LGB (100 μM) with/without dye **VB1Cl** (150 μM) in PBS solution (pH 7.2, 0.1 M).



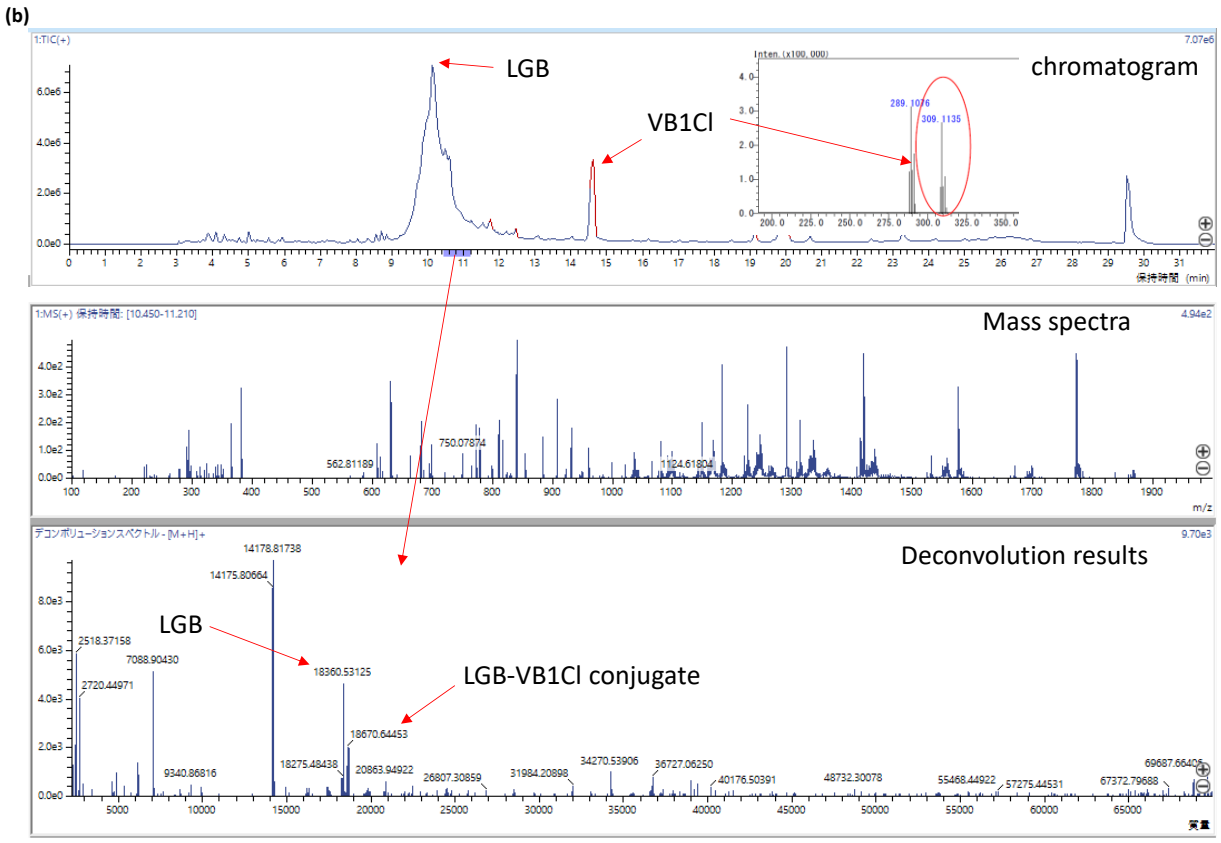


Figure S12. Mass spectrometry on unfolded protein β-lactoglobulin (LGB) with/without **VB1Cl** labelling. (a) LGB only, found 18361.53516. (b) VB1Cl-LGB conjugate, found 18670.64453 (one **VB1Cl** adduct), **VB1Cl** was found at 309.1135.

1.15. Selectivity of VB2Cl towards different amino acids

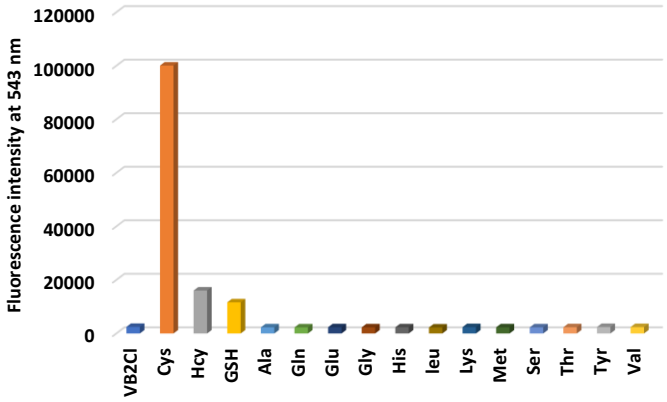


Figure S13. Fluorescence spectra and quantified intensity of **VB2Cl** (5 μM) at 543 nm upon addition of varied analytes (1 mM) after incubated for 1 h in MeCN/PBS (pH 7.2, v/v=1/1). Excitation wavelength is 480 nm.

1.16. Cytotoxicity Assay (MTT Assay)

HeLa human cancer cells were cultured at 37 ˚C and 5% CO_2 in DMEM supplemented with 10% fetal bovine serum (FBS), 500 units Penicillin and 500 µg Streptomycin. Measurement of cell viability was evaluated by reducing of MTT to formazan crystals using mitochondrial dehydrogenases. The cytotoxicity assays were performed in 96-well plates with a seeding density of 1×10^4 cells per well. After 24 h of cell attachment at 37 ˚C in 5% CO_2 before adding test substances. The plate was washed with 100 µL/well PBS. Then the cells were cultured in medium with 0.01, 0.1, 1, 10 µM of probe **VB1Cl** or **VB2Cl** for 24 h. Cells in culture medium without probe were used as the control. Six replicate wells were used for each control and test concentration. 100 µL of MTT (0.5 mg/mL) prepared in medium was added to each well and the plates were incubated at 37 °C for another 4 h in a 5% CO_2 humidified incubator. Wells without cell were used as the blank. The medium was carefully removed and washed with PBS, and the purple crystals were lysed in 200 µL DMSO. Absorbance at 550 nm was measured with micro-plate spectrophotometer (800TS, Biotek). Cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = (A_{test}-A_{blank})/(A_{control}-A_{blank}) \times 100$$

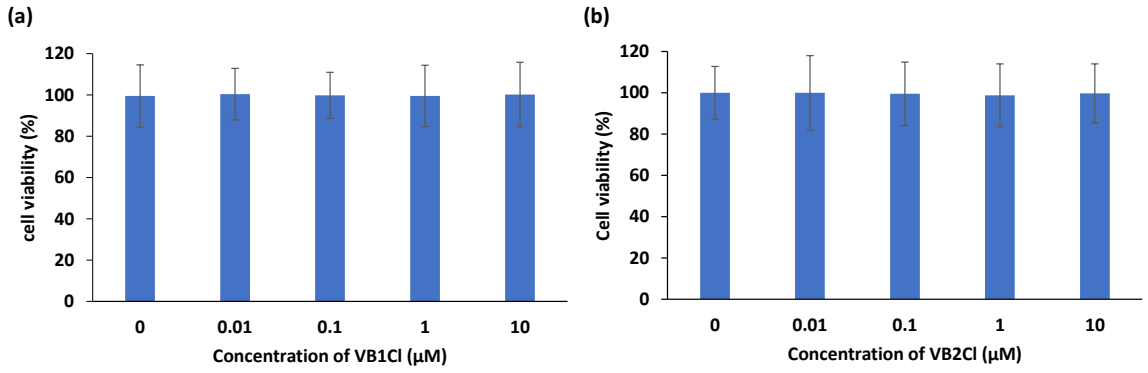


Figure S14. Cytotoxicity assays of (a)**VB1Cl** and (b) **VB2Cl** at 0, 0.01, 0.1, 1, 10, and 100 µM against HeLa cells for 24 h.

1.17. Living cell imaging

HeLa Cells (1×10^5 cells) were seeded in high µ-35 mm dish (ibidi) and incubated for 24 h at 37 °C under 5% CO_2. **VB1Cl** or **VB2Cl** (5 µM) was added to the dishes after washed with DMEM without phenol red (1 mL × 2) and cells were further incubated for 1 h at 37 °C under 5% CO_2, followed by washing thrice with phosphate-buffered saline (PBS). For the thiol-blocking experiment, HeLa cells were precultured with

N-ethylmaleimide (NEM, 2 mM) for 30 min, and then treated with **VB1Cl** or **VB2Cl** (5 μM) for 1 h, followed by washing thrice with phosphate-buffered saline (PBS). The fluorescence imaging was performed with LSM 700 inverted fluorescence microscope with 63× objective lens. Under the confocal fluorescence microscope, **VB1Cl** and **VB2Cl** was excited at 488 nm and emission was collected at 490–540 nm and 490–550 nm. For the colocalization experiments, the cells were treated with MitoTracker Deep Red (200 nM) or LysoTracker Deep Red (50 nM) or ER-Tracker Red (1 μM) for 15 mim. The intracellular localization of these reagents was analyzed by CLSM. For fixation assays,[4] labeled HeLa cells were washed with phosphate-buffered saline (PBS (–)), and fixed with methanol at –20 °C for 15 min. Methanol was replaced with PBS (–), and the intracellular localization of these reagents was analyzed by confocal imaging. For visualization of stress-induced unfolded proteins, HeLa cells were pretreated with tunicamycin (5 μg/mL for 8 h) or MG132 (0.5 μg/mL for 4 h) at 37 °C under 5% CO_2, followed by staining with **VB1Cl** (5 μM) for 1 h, then labelled cells for confocal imaging after fixation by cold methanol. The fluorescence intensities were extracted from seven POIs in each image. Statistical analyses were performed with a t-test (n = 5) relative to the data of no stress-treated cells. Conditions: for **VB1Cl**, λ_{ex}= 488 nm, λ_{em} = 490–540 nm. For LysoTracker Deep Red, λ_{ex}= 633 nm, λ_{em} = 640–720 nm. For ER-Tracker Red, λ_{ex}= 561 nm, λ_{em} = 565–640 nm. For Mito-Tracker Deep Red, λ_{ex}= 633 nm, λ_{em} = 640–760 nm.

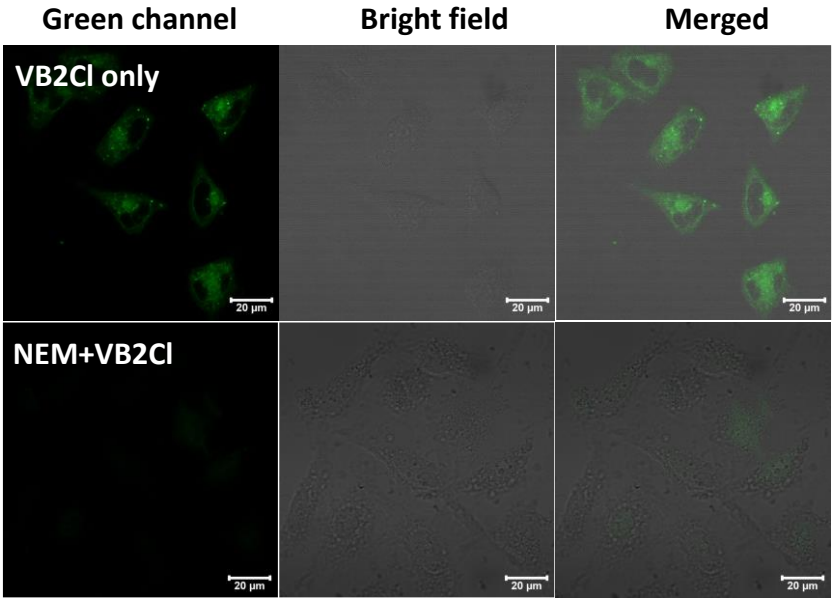


Figure S15. Confocal images in living HeLa cells. HeLa cells were pretreated with or without NEM (*N*-ethylmaleimide, 2 mM) for 30 min, then washed with DMEM and treated with probe **VB2Cl** (5 μM) for 1 h. After washed with DMEM for 2 times, fluorescence images were acquired by confocal microscopy. Green channel: 490–550 nm. λ_{ex} = 488 nm. Scale bar: 20 μm.

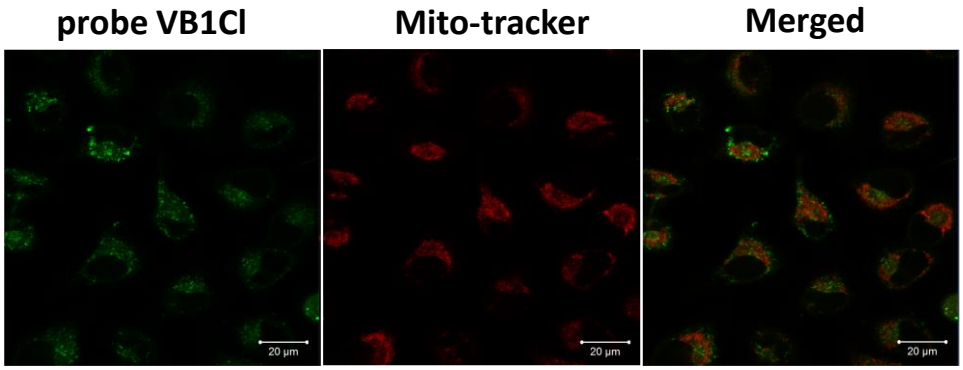


Figure S16. Representative confocal merged images of living HeLa cells treated with 5 μM of **VB1Cl** for 1 h and MitoTracker Deep Red (200 nM) for 15 min. Conditions: for **VB1Cl**, λ_{ex}= 488 nm, λ_{em} = 490–540 nm. For Mito-Tracker Deep Red, λ_{ex}= 633 nm, λ_{em} = 640–710 nm.

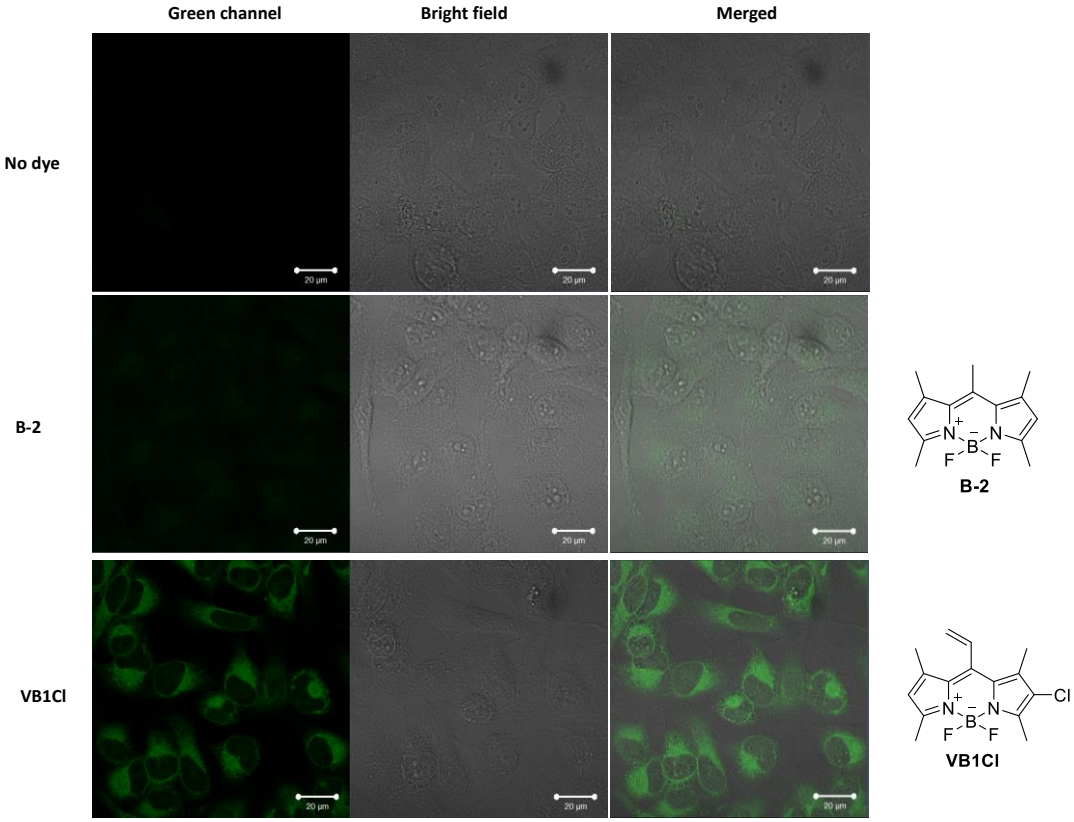


Figure S17. Confocal microscopy images of HeLa cells incubated with probe **VB1Cl** or **B-2** (5 μM) for 90 min, then fixation of cells by MeOH for 15 min. Excitation at 488 nm.

2. References

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(2) P. G. Seybold, M. Gouterman and J. Callis, *Photochem. Photobiol.*, 1969, **9**, 229–242.

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(4) A. Fujisawa, T. Tamura, Y. Yasueda, K. Kuwata and I. Hamachi, *J. Am. Chem. Soc.* 2018, **140**, 17060–17070.

<Cartesian coordinates for optimized geometry of VB1Cl and VB1Cl-Cys>

The ground state of VB1Cl optimized at the B3LYP/6-31G(d) level

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The excited state of VB1Cl optimized at the CAM-B3LYP/6-31G(d) level

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The ground state of VB1Cl-Cys adduct optimized at the B3LYP/6-31G(d) level

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The excited state of VB1Cl-Cys adduct optimized at the CAM-B3LYP/6-31G(d) level

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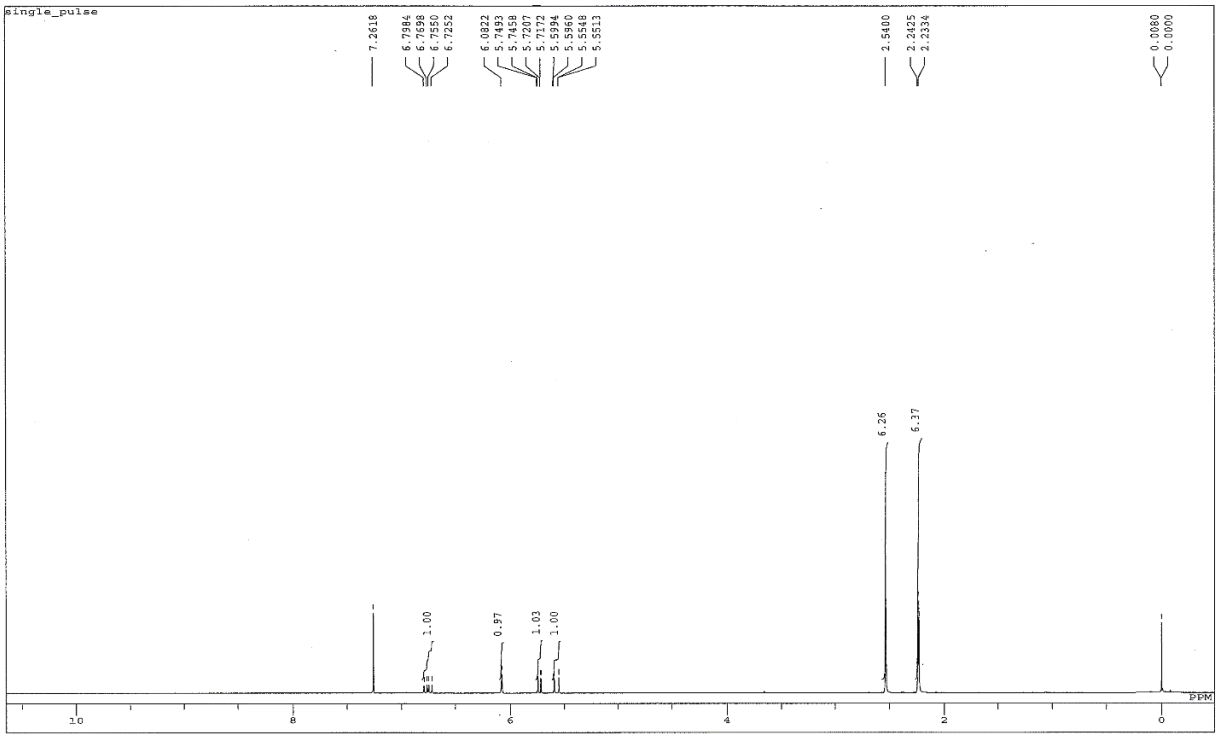
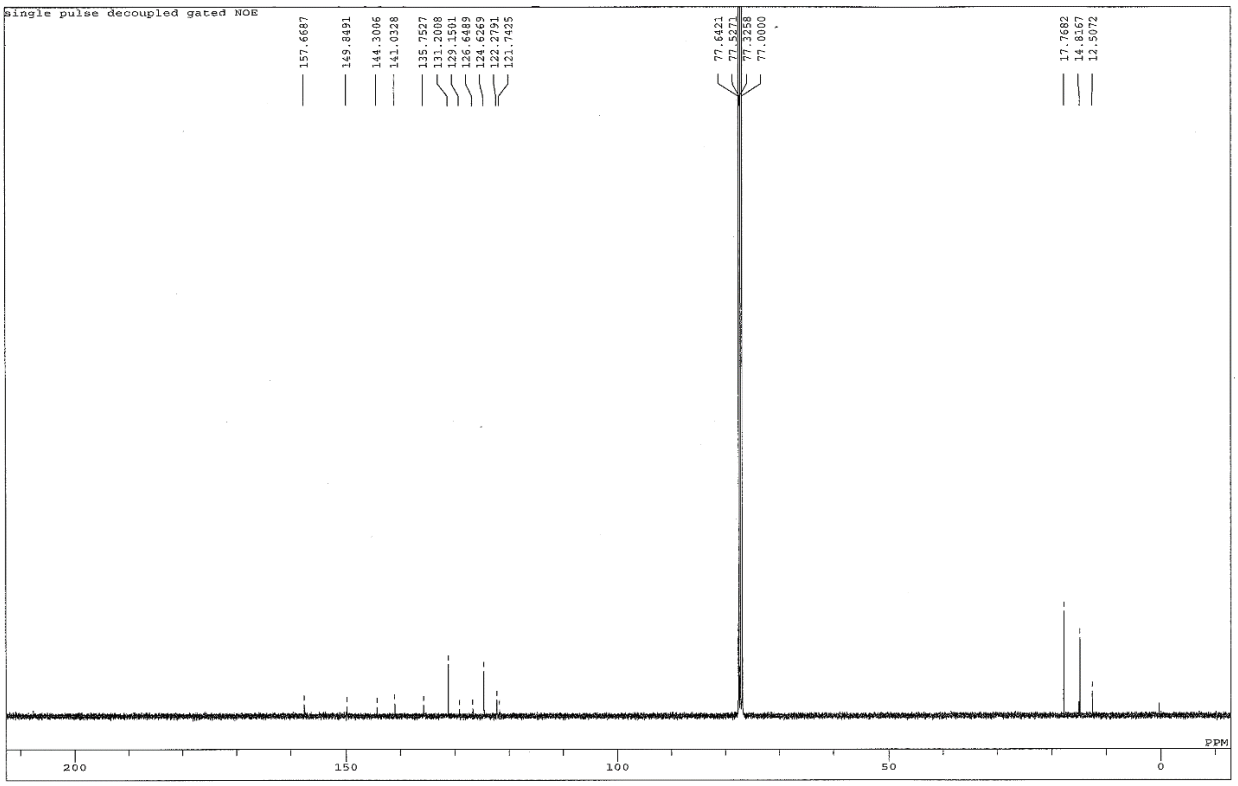
3. NMR spectra

^{1}H NMR of **VB1Cl** ($CDCl_3$)



^{13}C NMR of **VB1Cl** ($CDCl_3$)



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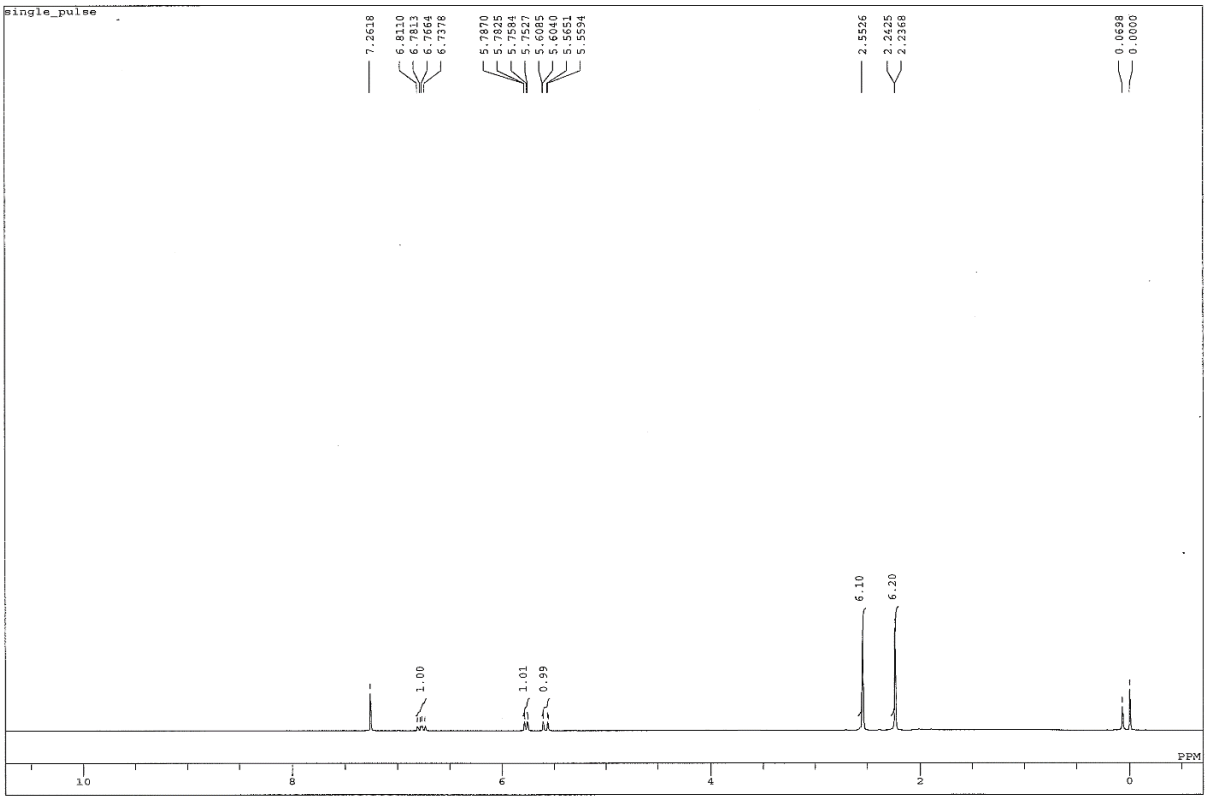

^{13}C NMR of **VB2Cl** ($CDCl_3$)