Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for

A Water-Soluble Sulfonate-BODIPY Based Fluorescent Probe for Selective Detection of HOCl/OCl^- in Aqueous Media

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Experimentals

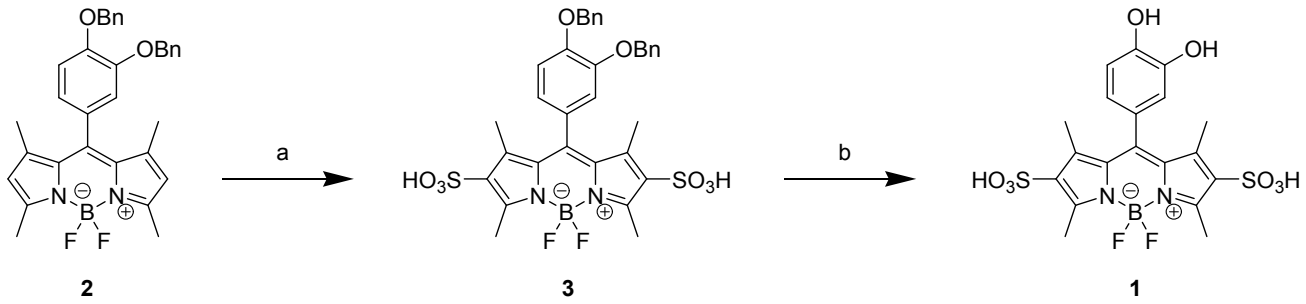
Materials

All reagents were of the highest commercial quality and used as received without further purification. All so lvents were spectral grade unless otherwise noted. Anhydrous dimethylformamide, tetrahydrofuran, and met hanol were obtained as a sure-seal bottle from Aldrich Co. Inc. (Milwaukee, WI). Silica gel (40 μm)was obt ained from Merck Inc. Aqueous solutions were freshly prepared with deionized water from a water purificati on system (Human Corp. Korea). Sulfur trioxide-pyridine complex was obtained from Aldrich Co. Inc. (Mil waukee, WI). Compound **2** was prepared in 49% yield, according to literature procedures.[1]

General methods, instrumentation and measurements

Synthetic manipulations that required an inert atmosphere (where noted) were carried out under argon using standard Schlenk techniques. NMR (^{1}H and ^{13}C) spectra were recorded on JEOL400 MHz spectrometers. Th e ^{1}H and ^{13}C chemical shifts were reported as δ in units of parts per million (ppm), referenced to the residual solvent. Splitting patterns are denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). High-resolution fast atom bombardment (FAB) mass spectrum was obtained at national center for inter-university research facilities. Absorption spectra were obtained on a Optizen 2120UV. Fluorescence m easurements were recorded on a Hitachi F-7000 fluorescence spectrophotometer using 10 mm quartz cuvette s with a path length of 1 cm. The reaction with various analytes was measured by monitoring changes in fluo rescence intensity upon addition of each analyte using a Synergy Mx Microplate Reader (BioTek, USA).

1. Synthesis



Scheme S1. Synthetic scheme of probe **1.** (a) sulfur trioxide-pyridine complex, DMF, 24 h, 60 °C, 93%; (b) 10% Pd/C, THF/MeOH, 12 h, 25 °C, 92%.

Compound 3: To a stirred solution of **2**[1] (50 mg, 0.093 mmol) in DMF (5 mL) at room temperature under ar gon atmosphere was added sulfur trioxide-pyridine complex (296 mg, 1.86 mmol). The reaction mixture was stirred for 24 hours at 60 °C. The reaction was stopped and cooled to room temperature. Following the remo val of the solvent under reduced pressure, the crude product was purified by preparative thin-layer chromato graphy (PTLC) using 5:1 dichloromethane : methanol as the mobile phase to afford **3** as a orange solid. (60. 2 mg, 93%).; ^{1}H-NMR (400 MHz, CD_3OD): δ = 7.39 (m, 10H), 7.18 (d, J = 8 Hz, 1H), 6.98 (s, 1H), 6.81 (d, J = 8 Hz, 1H), 5.24 (s, 2H), 5.18 (s, 2H), 2.77 (s, 6H), 1.63 (s, 6H); ^{13}C-NMR (100 MHz, CD_3OD): δ = 150.0 , 149.8, 145.1, 142.2, 136.9, 136.8, 134.4, 130.5, 128.2, 128.1, 127.8, 127.7, 127.6, 127.4, 127.3, 120.8, 116. 1, 114.8, 71.0, 70.8, 13.1, 12.1; HR-MS (FAB): calcd. for $C_{33}H_{30}BF_2N_2NaO_8S_2$ $[M+Na]^-$ 741.1300, found 74 1.1298.

Probe 1: To a 100 mL schlenk flask with stiring bar was added **3** (150 mg, 0.215 mmol), 10% Pd/C (56 mol%) and MeOH/THF (21 mL) under a H_2 atmosphere (1 atm) at room temperature. After the reaction mixture w as stirred for 12 hours, the resulting mixture was filtered through Celite and the filter cake was washed with MeOH and THF. Following the removal of the solvent under reduced pressure, the crude product was purifi

ed by column chromatography on silica gel using 3:1 dichloromethane : methanol as the mobile phase to aff ord probe **1** as a orange solid. (102.5 mg, 92% yield).; ^{1}H-NMR (400 MHz, CD_3OD): δ = 6.95 (d, J = 7.6 Hz, 1H), 6.69 (s, 1H), 6.59 (d, J = 7.6 Hz, 1H), 2.78 (s, 6H), 1.83 (s, 6H); ^{13}C-NMR (100 MHz, CD_3OD): δ = 154.8, 146.7, 146.6, 146.1, 142.2, 134.2, 130.7, 125.4, 119.1, 116.1, 114.8, 13.0, 12.1; HR-MS (FAB): cal cd. for $C_{19}H_{18}BF_2N_2NaO_8S_2$ $[M+Na]^-$ 561.0361, found 561.0364.

2. Studies of Photophysical Properties

Table S1. Photophysical properties of probe **1**

Compound	Solvent	$\lambda_{abs,max}$, nm	ε, $M^{-1}cm^{-1}$	$\lambda_{em,max}$, nm	Φ_{FL}[b]
1	Buffer[a]	497	67960	519	0.001
1	H_2O	497	70330	518	0.001
1	C_2H_5OH	504	68410	523	0.003

[a]Data was obtained in sodium phosphate buffer (10 mM, pH 7.8). Excited at 460 nm for **1**. [b]Quantum yield v s. Fluorescein in 0.1 N NaOH (Φ_F = 0.95).[2]

(a) Absorption and emission spectra of probe 1

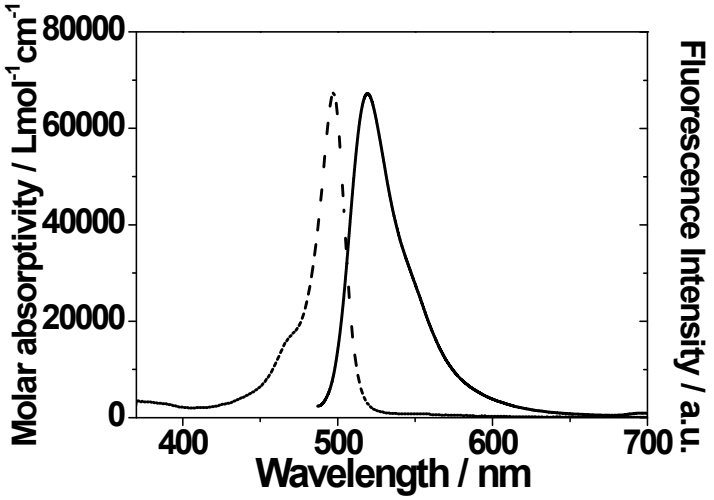


Figure S1. Absorption (dash-line) and emission (solid-line) spectra of probe **1** in phosphate buffer (10 mM, pH = 7.8, 25 °C). Excited at 460 nm. [**1**] = 10 μM.

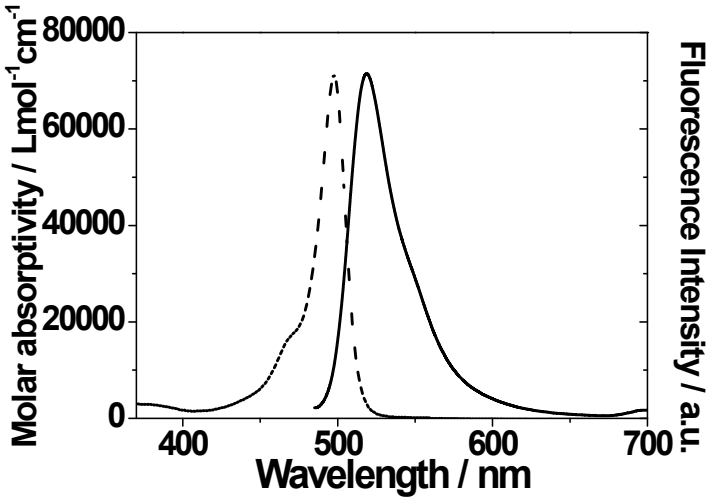


Figure S2. Absorption (dash-line) and emission (solid-line) spectra of probe **1** in H_2O (25 °C). Excited at 46 0 nm. [**1**] = 10 μM.

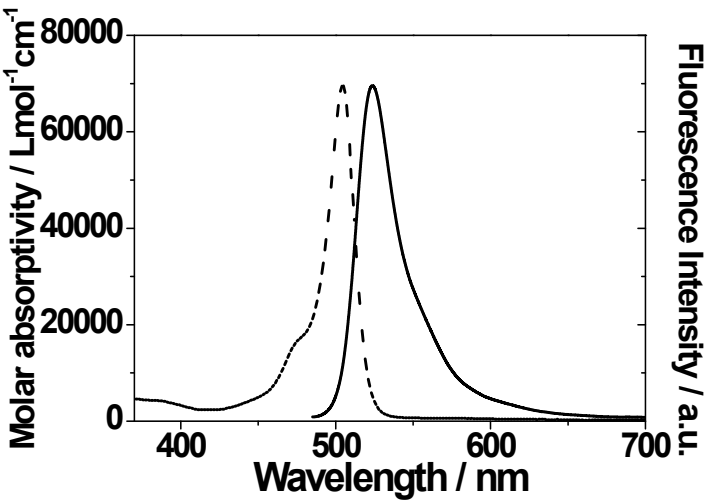


Figure S3. Absorption (dash-line) and emission (solid-line) spectra of probe **1** in EtOH (25 ^{o}C). Excited at 4 60 nm. [**1**] = 10 μM.

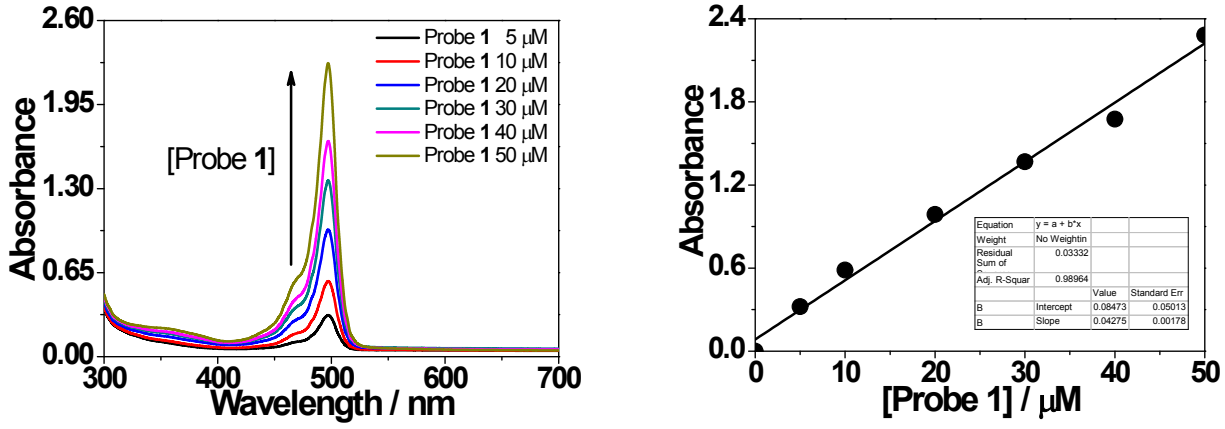


Figure S4. (left) Absorption spectra of probe **1** at various concentrations (5 - 50 μM) in H_2O (25 °C). (right) A plot of absorbance at 497 nm as a function of [probe **1**] in H_2O.

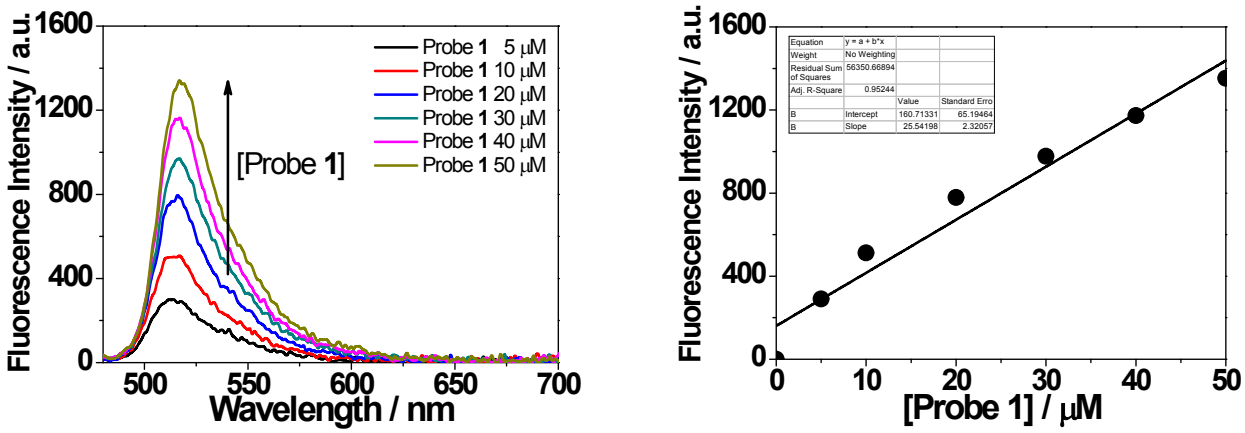


Figure S5. (left) Emission spectra of probe **1** at various concentrations (5 - 50 μM) in H_2O (25 °C). (right) A plot of emission intesities at 518 nm as a function of [probe **1**] in H_2O. Excited at 460 nm.

(b) Photostability studies

Photostability of probe **1** was investigated by continuous irradiation of **1** (10 μM) dissolved in phosphate buf fer at 25 °C using 150 W steady-state Xe lamp as the light source under aerobic conditions. The photoinduce d degradation was quantified by monitoring the decrease of fluorescence intensity of **1** as a function of irradi ation time.

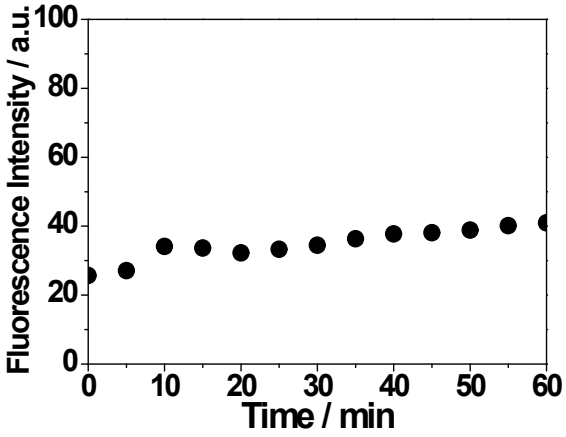


Figure S6. Photostability of probe **1** in phosphate buffer (10 mM, pH 7.8) at 25 °C. Fluorescence intensity w as measured at 519 nm. Excited at 460 nm. [**1**] = 10 μM.

(c) Chemical stability studies

To investigate the stability of probe **1**, fluorescence spectra of probe **1** in aerated assay solution was recorded every 30 min for 3 hours. The chemical stability of probe **1** was quantified by monitoring the fluorescence intensity.

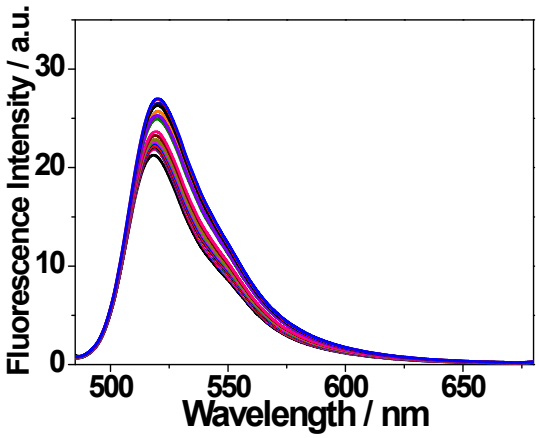


Figure S7. Chemical stability of probe **1** (10 μM) in phosphate buffer (10 mM, pH 7.8) at 25 °C. The emission spectra were obtained every 2 min (0 - 1 hr). Excited at 460 nm.

3. Fluorometric Assay Studies

(a)Fluorescence turn-on response of probe 1 to NaOCl

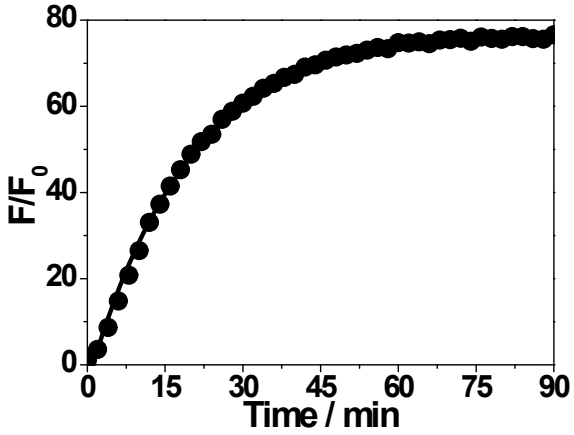


Figure S8. A plot of relative fluorescence intensity (F/F_0) at 525 nm in the presence of NaOCl (40 μM) as a function of incubation time. Excited at 460 nm. F_0 and F correspond to the fluorescence intensity of probe **1** in the absence and the presence of NaOCl, respectively. [**1**] = 10 μM.

(b) pH Effect on probe 1 in the absence and the presence of NaOCl

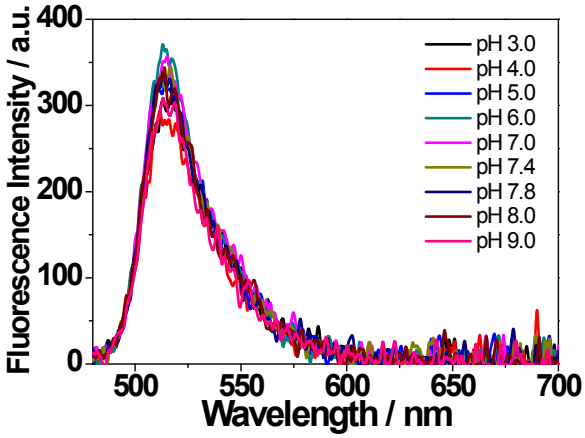


Figure S9. Fluorescence emission spectra of probe **1** in different pH conditions at 25 °C. Excited at 460 nm. [**1**]= 10 μM.

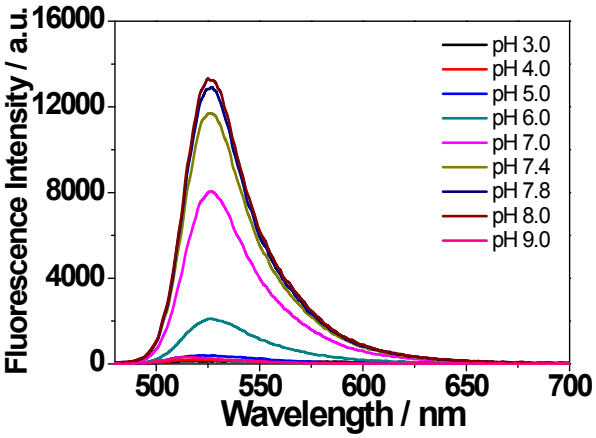


Figure S10. Fluorescence emission spectra of probe **1** upon addition of NaOCl (Incubation time = 60 min) i n different pH conditions at 25 °C. Excited at 460 nm. [**1**]= 10 μM. [NaOCl] = 40 μM.

(c) NaOCl concentration-dependent fluorescence response of probe 1

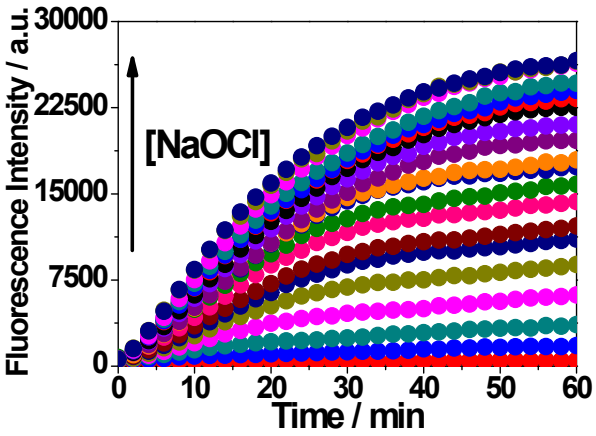


Figure S11. Fluorescence response of probe **1** (10 μM) upon incubation with different amounts of NaOCl (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 μM) in phosphate buffer (10 mM, pH = 7.8, 25 °C). The spectra were obtained every 30 sec (0 – 60 min) after the addition of NaOCl, and fluorescence intensity at 525 nm was recorded. Excited at 460 nm.

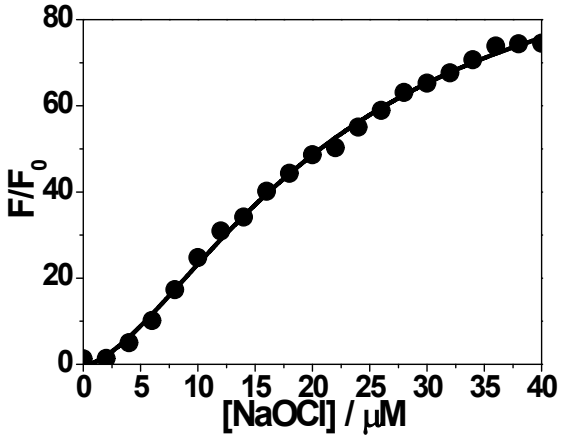


Figure S12. A plot of relative fluorescence intensity (F/F_0) at 525 nm as a function of [NaOCl]. Incubation time = 60 min. F_0 and F correspond to the fluorescence intensity of probe **1** in the absence and the presence of NaOCl, respectively.

(d) Absorption and emission spectra probe 1 in the presence of bovin serum albumin (BSA)

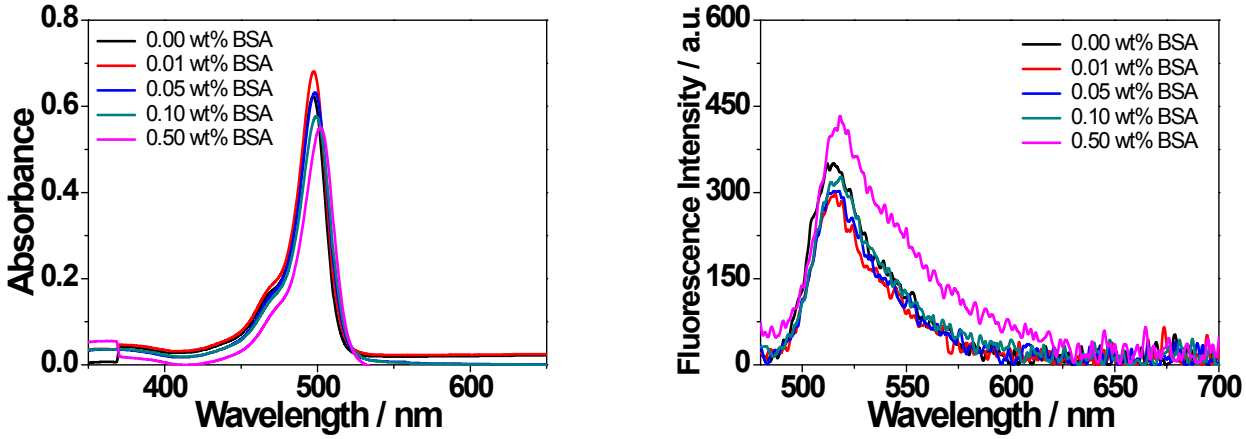


Figure S13. Absorption (left) and emission spectra (right) of probe **1** (10 μM) depending on BSA fraction in phosphate buffer (10 mM, pH = 7.8, 25 °C). Excited at 460 nm.

(e) Fluorescence response of probe 1 to various analytes

A solution of probe **1** (0.1 mM in water, 20 μL) was diluted in phosphate buffer (10 mM, pH = 7.8, 160 μL) at 25 °C. Each analyte was prepared as concentration of 1 mM in deionized water. Analytes were added to pr obe solution in 96-well flat bottom microplates, and the reactions were monitored at 25 °C for 60 minutes. T he fluorescence signal for each well was measured at 525 nm (λ_{ex} = 460 nm). Various reactive oxygen speci es (ROS) were prepared as follows: Superoxide ($\bullet O_2^-$) was added as solid KO_2. Hydrogen peroxide (H_2O_2), *t ert*-butyl hydroperoxide (TBHP), sodium hypochlorite (NaOCl) were delivered from 30%, 70%, and 5% aqu eous solutions, respectively. Hydroxyl radical (•OH) and *tert*-butoxy radical (•O^{t}Bu) were generated by Fent on reaction of 1 mM Fe^{2+} with 200 μM H_2O_2 or 200 μM TBHP, respectively. Peroxynitrite solution was syn thesized as reported.[3] The peroxynitrite concentration was estimated by using an extinction coefficient of 16 70 ± 50 cm^{-1} $(mol/L)^{-1}$ at 302 nm.[4] Experiments employed 40 μM NaOCl, 40 μM peroxynitrite, and 100 μ M for all other ROS.

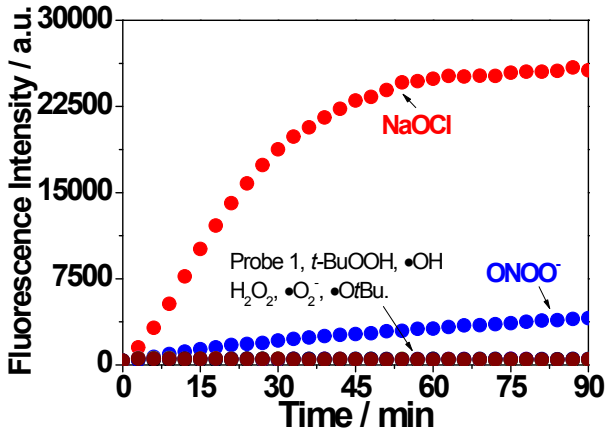


Figure S14. Time-dependant fluorescence changes at 525 nm of probe **1** (10 μM) upon addition of each anal yte (40 μM NaOCl, 40 μM $ONOO^-$, and 100 μM for others). All the data were obtained in phosphate buffer (10 mM, pH=7.8) at 25 °C. Excited at 460 nm.

4. Kinetics Studies

Determination of kinetic constant: Pseudo-first Order Kinetics

Rate constants were determined for the reaction between probe **1** and NaOCl in phosphate buffer (10 mM, p H = 7.8, 25 °C). Linear calibration of the fluorescence signal against the concentration of product produced f rom the reaction of probe **1** with NaOCl was carried under the assumption that probe **1** (1 μM) is completely converted to product in the presence of a large excess of NaOCl (40 μM) and therefore the fluorescence sign al at saturation corresponds to [product] = 1 μM. Fluorescence data were modeled (least squares curve fittin g) against eq. 1 (see below) where the parameter *a* is added to account for delay between the addition of the reagents and the time at which the first measurement can be acquired due to experimental limitation. The pse udo-first order rate constants (k_{obs}) obtained 0.11077 min^{-1} for 40 μM NaOCl. These correspond to second or der rate constant (k) of 2769.25 M^{-1} min^{-1} after division by the concentration of analyte.

$$f(t) = 1\ \mu M\left[1 - exp(-k_{obs}(t + a))\right] \qquad \text{eq.1}$$

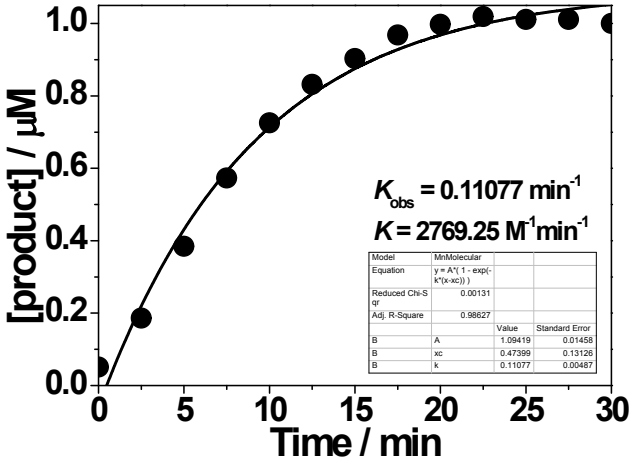


Figure S15. Pseudo-first order kinetics for the fluorescence response of probe **1** (1 μM) upon incubation wit h NaOCl (40 μM) in phosphate buffer (10 mM, pH = 7.8, 25 °C). Fluorescence intensity at 525 nm was meas ured. Excited at 460 nm.

5. Analysis of Reaction Product by LC-MS

The proposed detection scheme was confirmed by analyzing the crude reaction mixture of probe **1** with NaO Cl using HPLC-MS. The probe **1** (50 μM) was dissolved in deionized water, and then 4 equivalents of NaO Cl were added into probe solution. The reaction mixture was analyzed by HPLC-MS with a linear gradient e lution (eluent A/B = 80/20, A: deionized water, B: Methanol with 5 mM ammonium formate, flow rate 0.3 mL/min). The reaction mixture of probe **1** with NaOCl shows intense peak of chloroquinone-BODIPY (*m/z* 547.1), which is consistent with that of isolated product from assay mixture solution.

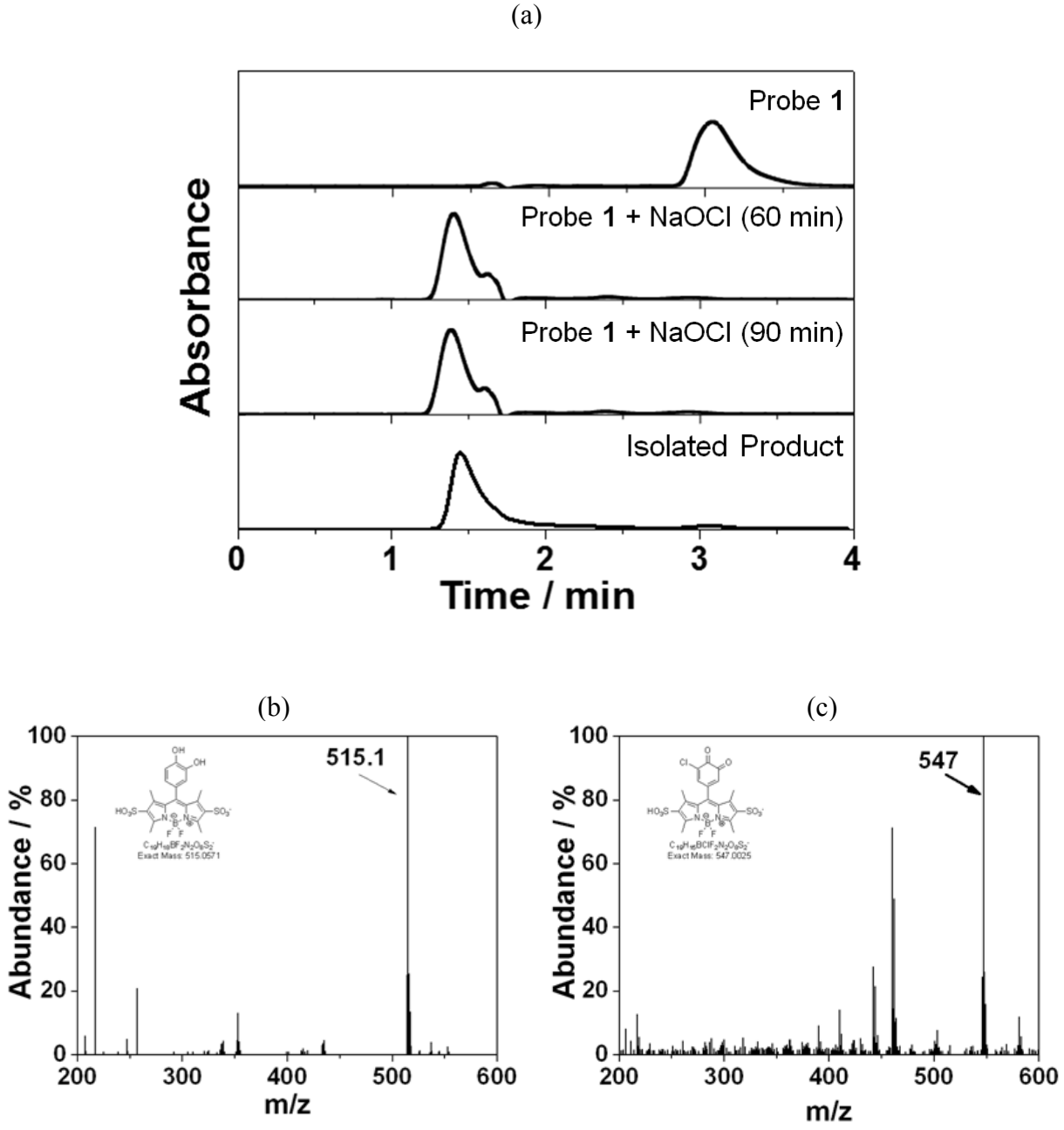
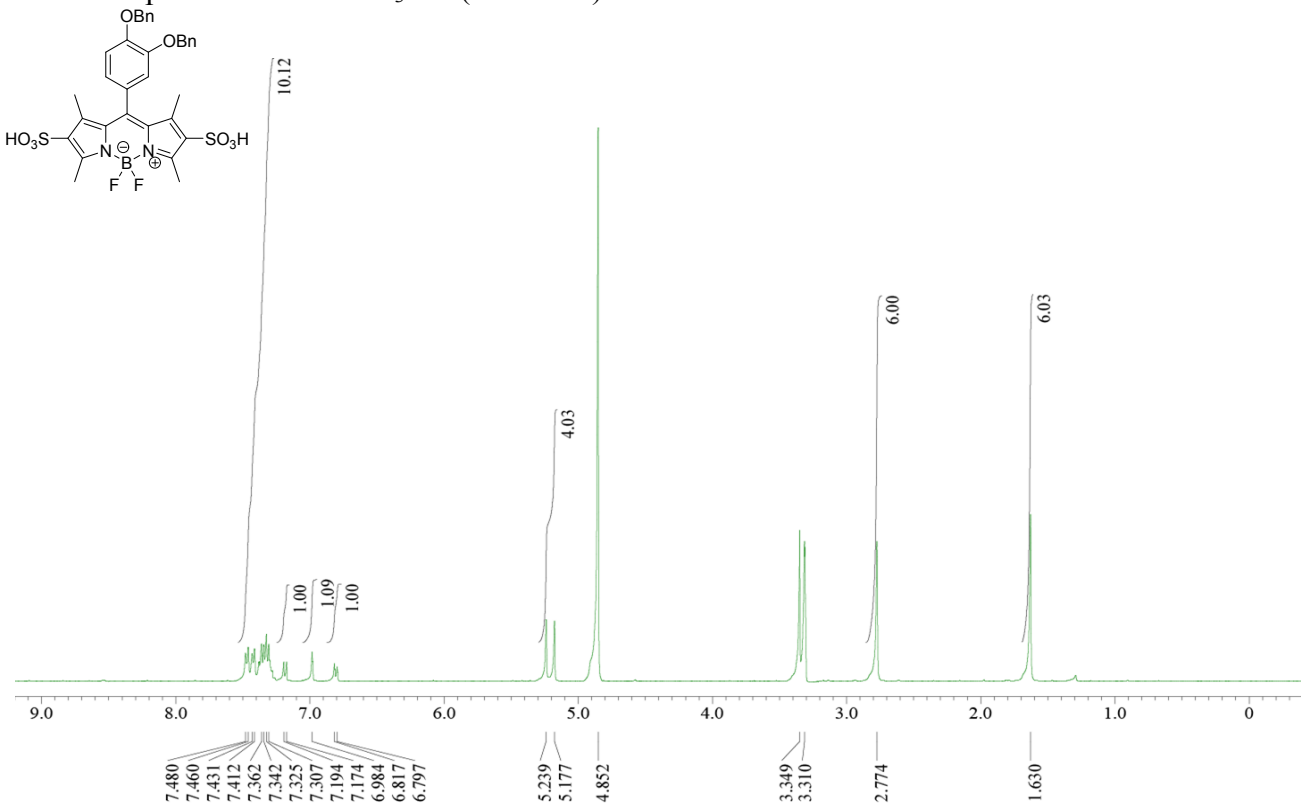


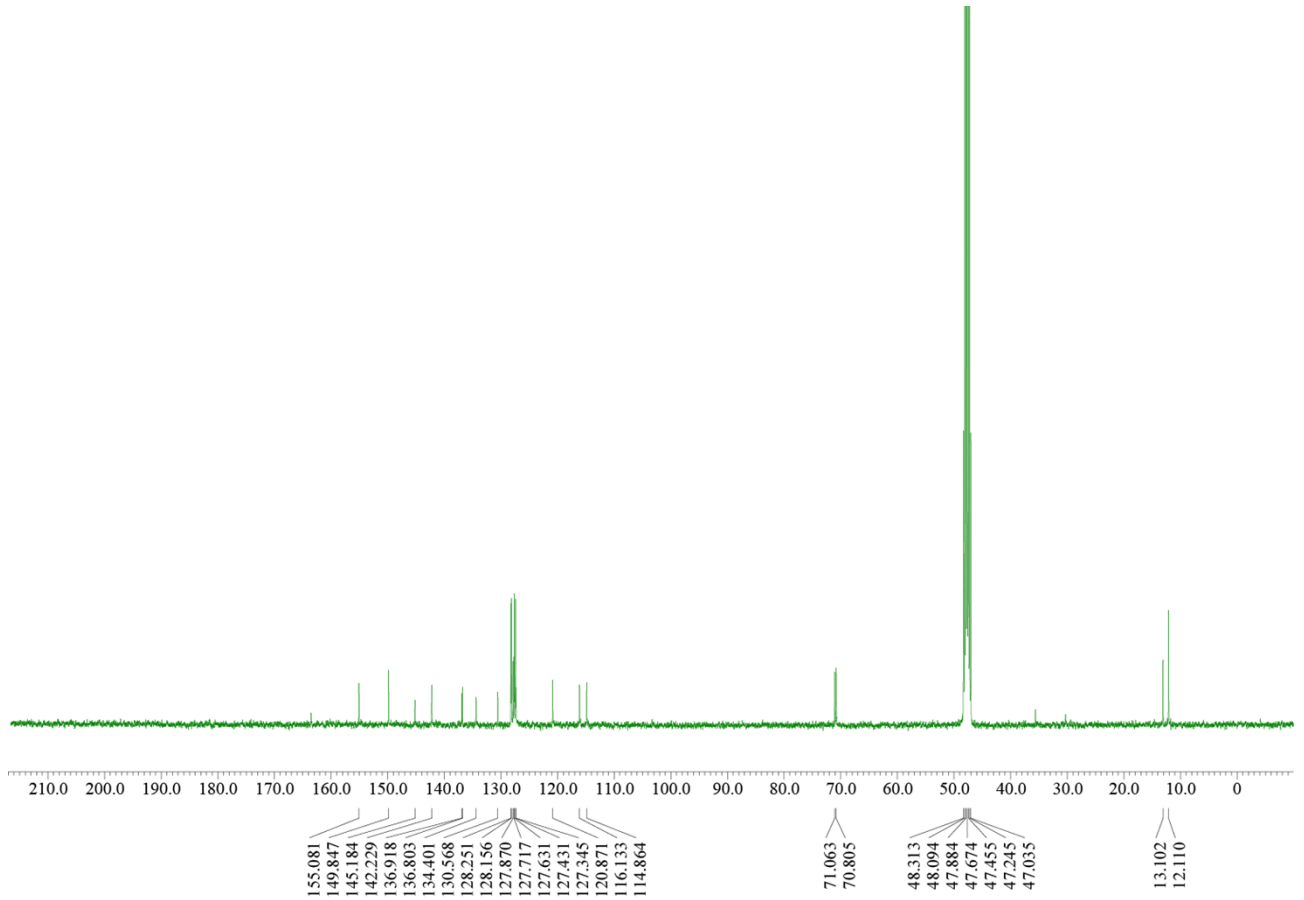
Figure S16. (a) HPLC chromatograms of probe **1** without NaOCl treatment (top); after NaOCl treatment in deionized water for 60 min and 90 min at 25 °C, respectively (middle); and isolated product only (bottom). ESI-MS spectra of the peak of retention time at 3.188 min (b) and 1.530 min (c). [**1**] = 50 μM, [NaOCl] = 20 0 μM.

6. ^{1}H-NMR and ^{13}C-NMR Spectra

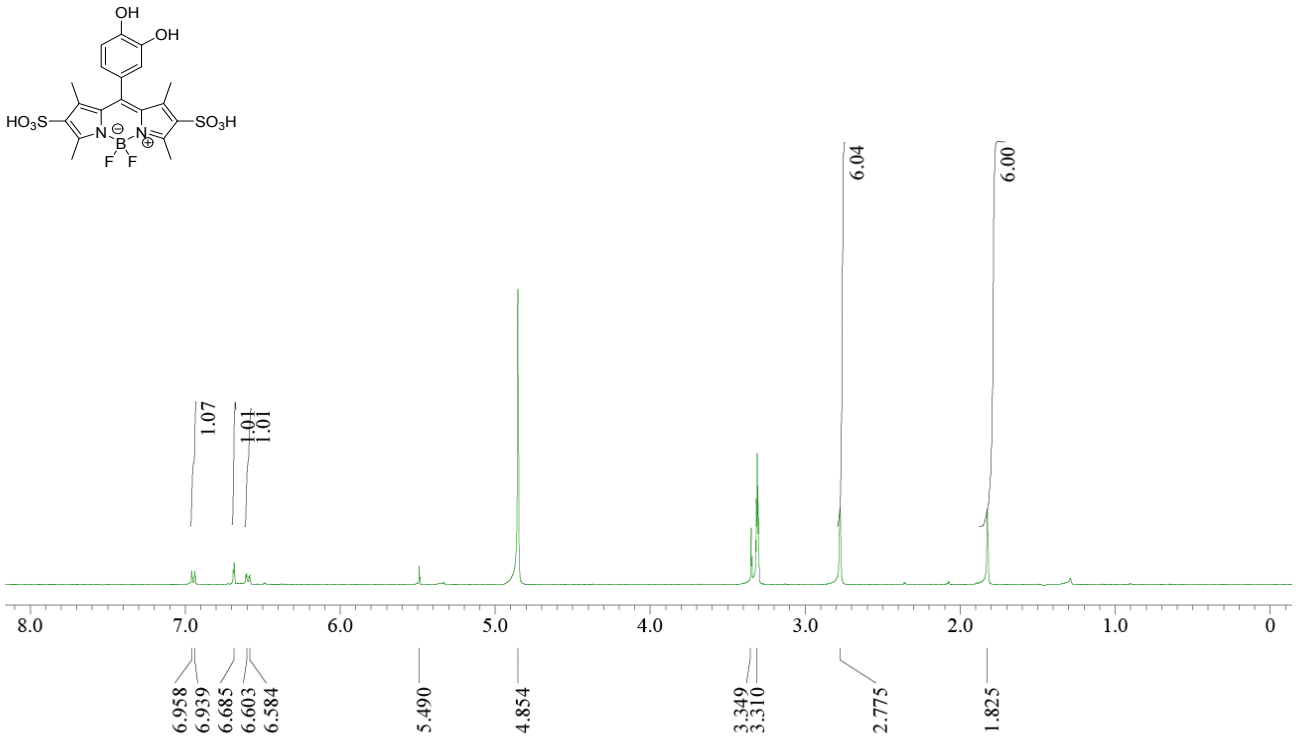
^{1}H-NMR spectrum of **3** in CD_3OD (400 MHz):



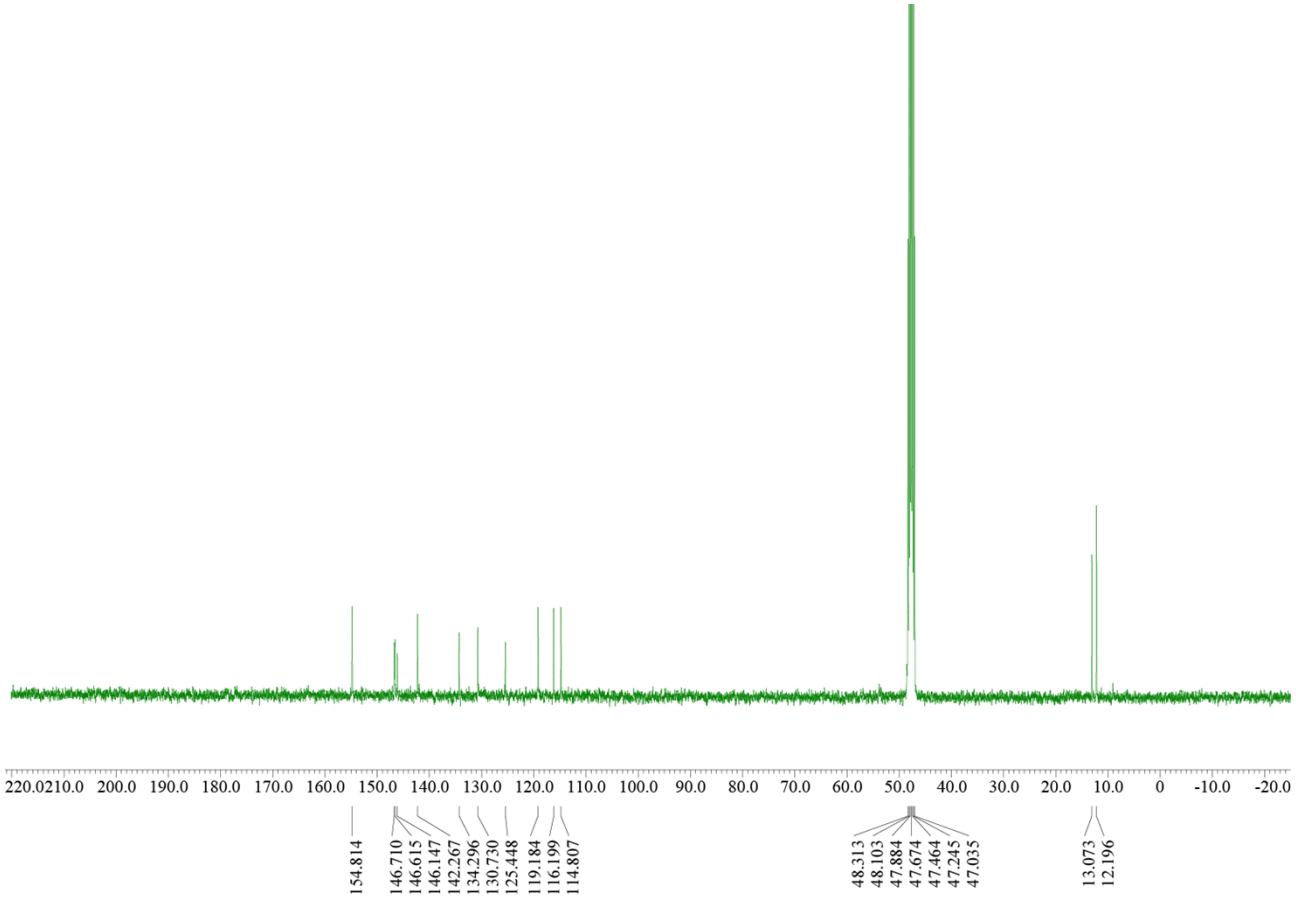
^{13}C-NMR spectrum of **3** in CD_3OD (100 MHz)



^{1}H-NMR spectrum of **1** in CD_3OD (400 MHz):



^{13}C-NMR spectrum of **1** in CD_3OD (100 MHz)



7. References

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4 M. N. Hughes and H. G. Nicklin, *J. Chem. Soc. (A)*, 1968, **2**, 450.