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Electronic Supplementary Information

Single molecular multianalyte signaling of sulfide and azide ions by a nitrobenzoxadiazole-based probe

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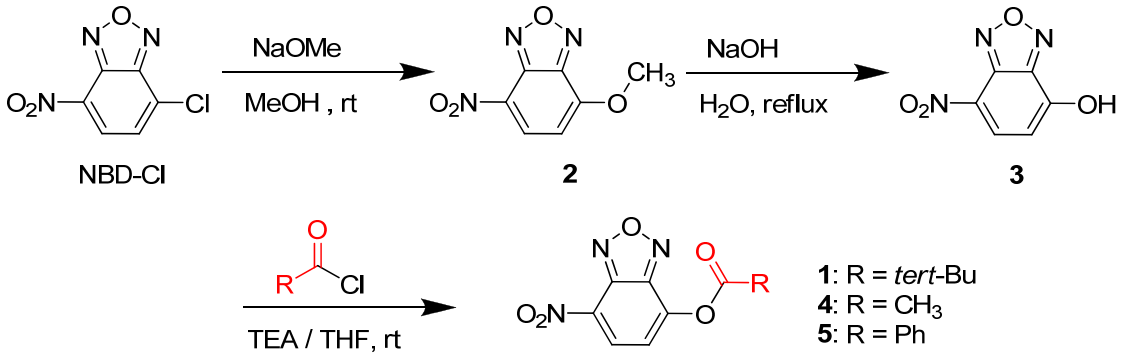
Contents

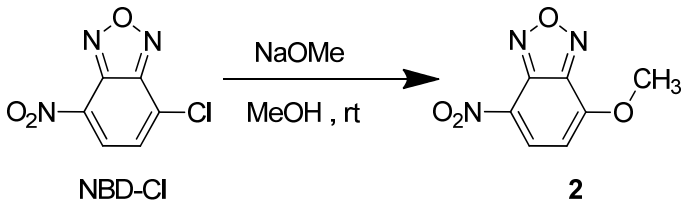
Experimental details.

General: 4-Chloro-7-nitrobenzofurazan (NBD–Cl), sodium methoxide, pivaloyl chloride, acetyl chloride, benzoyl chloride, sodium azide, and sodium sulfide were purchased from commercial source and used as received. All solvents were obtained as "spectroscopic grade". ^{1}H NMR and ^{13}C NMR spectra were measured on 600 MHz and 150 MHz spectrometer, respectively, and referenced to the residual solvent signals. UV–vis spectra were recorded with a spectrophotometer equipped with a Peltier temperature controller. Fluorescence spectra were measured with a steady-state spectrofluorometer. Mass spectra were obtained on a FAB mass spectrometer.



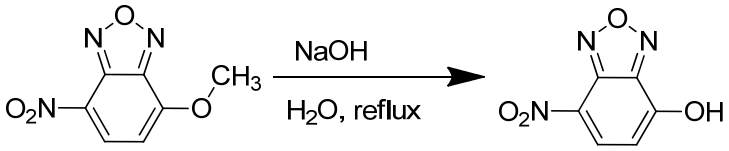
Preparation of NBD–OCH_3 2.

A mixture of sodium methoxide (0.22 g, 4.0 mmol) and NBD–Cl (0.2 g, 1.0 mmol) in methanol (15 mL) was stirred for 2 h at room temperature. The resulting solution was acidified by 1 N HCl to pH 2 and filtered. The residue was purified by column chromatography (silica gel 240 mesh, dichloromethane) to yield NBD–OCH_3 **2** (0.17 g, 87%) as a dark yellow powder. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.56 (d, J = 8.3 Hz, 1H), 6.70 (d, J = 8.3 Hz, 1H), 4.24 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.3, 145.2, 143.9, 133.8, 103.9, 103.8, 57.8; HRMS: (FAB^+); *m/z* calcd for $C_7H_6N_3O_4^+$ $[M+H]^+$: 196.0358, found 196.0361.



Preparation of NBD–OH 3.[1]

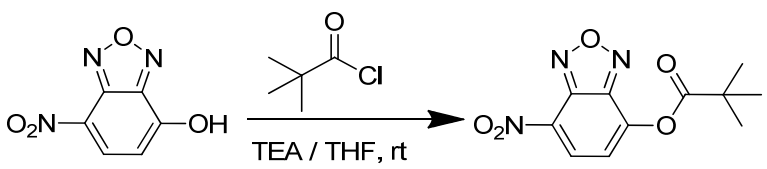
Sodium hydroxide (0.8 g, 20 mmol) was added to a solution of NBD–OCH_3 **2** (0.20 g, 1.0 mmol) in distilled water (100 mL). The mixture was refluxed for 3 h. The solution was cooled to room temperature and acidified by 1 N HCl to pH 2. The solution was extracted with diethyl ether. The organic solution was evaporated and the residue was purified by column chromatography (silica gel, 1st eluent = dichloromethane : methanol = 5 : 1, v/v, 2nd eluent = methanol) to give compound **3** (0.10 g, 60%) as a yellow powder. ^{1}H NMR (600 MHz, CD_3OD) δ 8.49 (d, J = 8.5 Hz, 1H), 6.51 (d, J = 8.5 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 175.0, 149.6, 147.5, 140.3, 116.0, 112.2; HRMS: (FAB^+); *m/z* calcd for $C_6H_3N_3O_4Na^+$ $[M+Na]^+$: 204.0021, found 204.0019.



2 **3**

Preparation of NBD–pivalate 1.

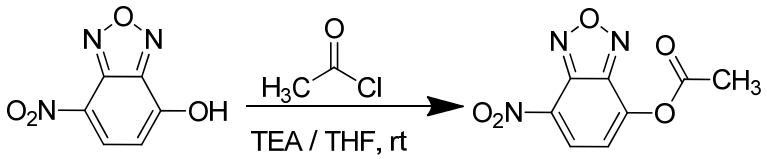
Pivaloyl chloride (0.37 mL, 3.0 mmol) was added to a solution of NBD–OH **3** (0.18 g, 1.0 mmol) and triethylamine (0.27 mL, 2.0 mmol) in THF (10 mL). The reaction mixture was stirred for 1 h at room temperature and then evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel, dichloromethane). The solid residue was crystallized from dichloromethane to give compound **1** (0.17 g, 65%) as a dark brown crystal. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.55 (d, J = 8.0 Hz, 1H), 7.44 (d, J = 8.0 Hz, 1H), 1.47 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.9, 145.9, 145.9, 143.8, 134.0, 131.6, 119.0, 39.9, 26.9; HRMS: (FAB^+); *m/z* calcd for $C_{11}H_{12}N_3O_5^+$ $[M+H]^+$: 266.0777, found 266.0775.



3 **1**

Preparation of NBD–acetate 4.

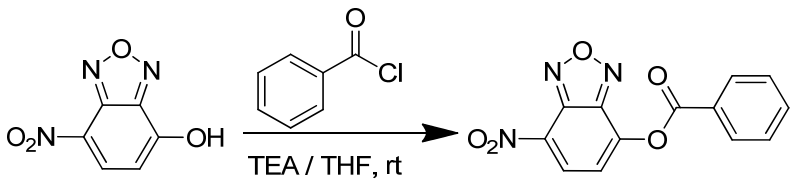
Compound **4** was prepared similarly by the reaction of NBD–OH **3** with acetyl chloride. The crude product was purified by column chromatography (silica gel, dichloromethane). The solid product was crystallized from dichloromethane to give compound **4** (0.10 g, 45%) as a brown crystal. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.55 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 2.52 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.9, 145.7, 145.0, 143.8, 134.1, 131.6, 119.0, 21.0; HRMS: (FAB$^+$); *m/z* calcd for $C_8H_6N_3O_5^+$ $[M+H]^+$: 224.0307, found 224.0308.



3 **4**

Preparation of NBD–benzoate 5.

Compound **5** was prepared likewise by the reaction of NBD–OH **3** with benzoyl chloride. The crude product was purified by column chromatography (silica gel, dichloromethane). The purified product was recrystallized from dichloromethane to give compound **5** (0.21 g, 75%) as a light yellow crystal. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.61 (d, J = 8.1 Hz, 1H), 8.29 (dd, J = 8.4 and 1.3 Hz, 2H), 7.75 (t, J = 7.5 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H), 7.62–7.58 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.8, 146.0, 145.4, 143.8, 135.1, 134.0, 131.8, 130.8, 129.1, 127.2, 119.0; HRMS: (FAB$^+$); *m/z* calcd for $C_{13}H_8N_3O_5^+$ $[M+H]^+$: 286.0464, found 286.0460.



3 **5**

Analysis of sulfide and azide ions in simulated wastewater.

Stock solutions of sulfide and azide ions (0.01 M) were prepared in simulated wastewater. Calculated amounts of stock solutions of sulfide, azide, HEPES buffer solution, and **1** were added to a vial, and the resulting solutions were diluted to 3.0 mL

with acetonitrile and wastewater. The final concentrations of **1**, HEPES buffer (pH 7.1), sulfide, and azide ions were 3.0×10^{-5} M, 1.0×10^{-2} M, 0–6.0×10^{-5}M, and 0–3.0×10^{-5} M, respectively, in a 1:1 (v/v) mixture of HEPES-buffered wastewater and acetonitrile. Simulated wastewater was prepared according to the reported composition of wastewater: $[Na^+] = 2.39 \times 10^{-3}$ M, $[K^+] = 2.81 \times 10^{-4}$ M, $[Mg^{2+}] = 2.88 \times 10^{-4}$ M, $[Ca^{2+}] = 2.50 \times 10^{-4}$ M, $[F^-] = 1.60 \times 10^{-5}$ M, $[Cl^-] = 9.87 \times 10^{-4}$ M, $[PO_4^{3-}] = 1.05 \times 10^{-4}$ M, $[SO_4^{2-}] = 2.08 \times 10^{-4}$ M, $[NO_3^-] = 4.84 \times 10^{-4}$ M, $[HCO_3^-] = 1.23 \times 10^{-3}$ M.[2]

Application of the probe 1 as a test strip.

The test strip was prepared by soaking filter paper (Whatman™ #.2) with the solution of probe **1** (1.0 mM, acetonitrile). Analytes containing sulfide or azide ions were dropped to the prepared test paper. After drying in air, pink colored spot (in sulfide signaling) or yellow colored fluorescence spot (in azide signaling) were developed. Optical images were recorded using a camera of iPhone 5S (Apple. Inc). Thus obtained pictures were analyzed with green or red channel values of image in Photoshop CS6 (Adobe Systems Incorporated).

[1] S. Fery-Forgues, D. Lavabre and J. Lozar, *New J. Chem.*, 1995, **19**, 1177.

[2] G. Tchobanoglous, F. L. Burton, *in Wastewater Engineering: Treatment Disposal Reuse*, McGraw-Hill, New York, 1991, p 1820.

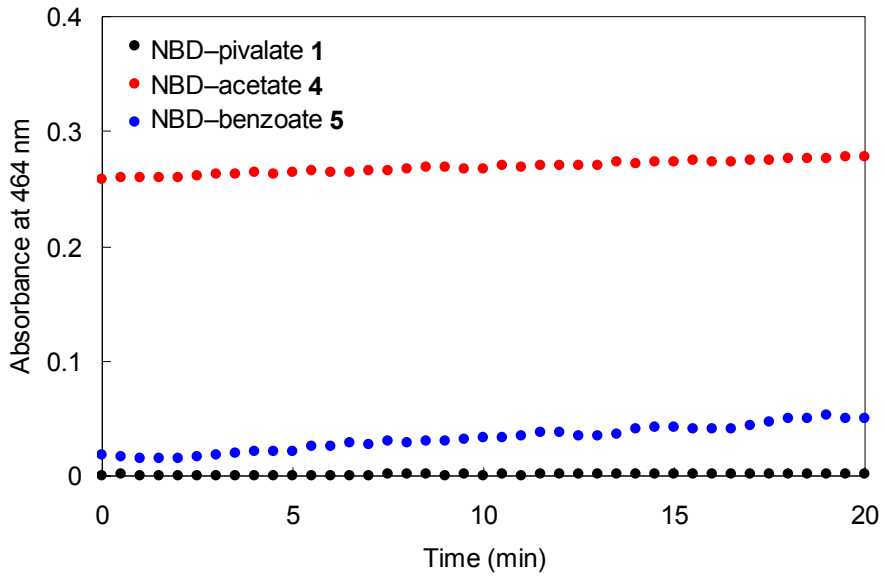


Fig. S1. Time trace for the changes in UV–vis absorbance of NBD–pivalate **1**, NBD–acetate **4**, and NBD–benzoate **5** at 464 nm. [**1**] = [**4**] = [**5**] = 3.0×10^{-5} M. In a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v).

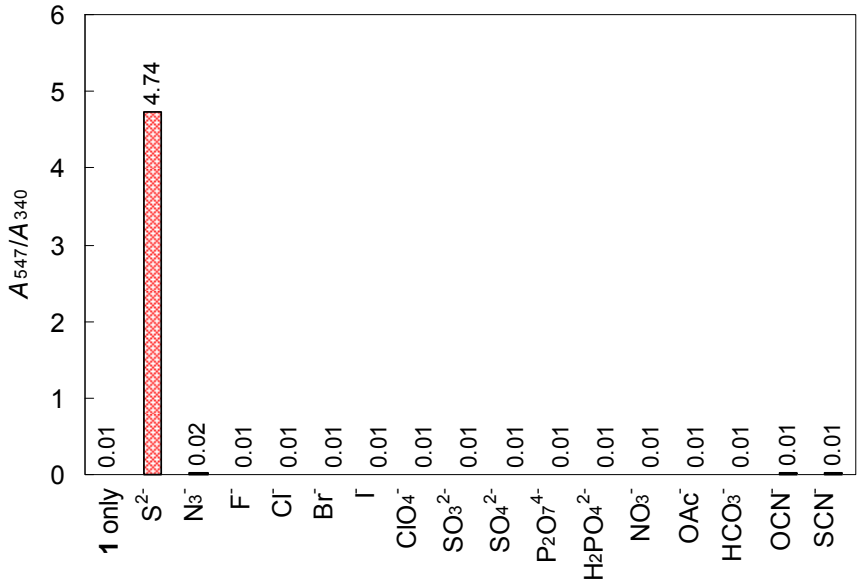


Fig. S2. UV–vis absorbance ratio at 547 nm and 340 nm (A_{547}/A_{340}) of **1** in the presence of representative anions. [**1**] = 3.0×10^{-5} M, [A^{n-}] = 3.0×10^{-4} M. In a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v).

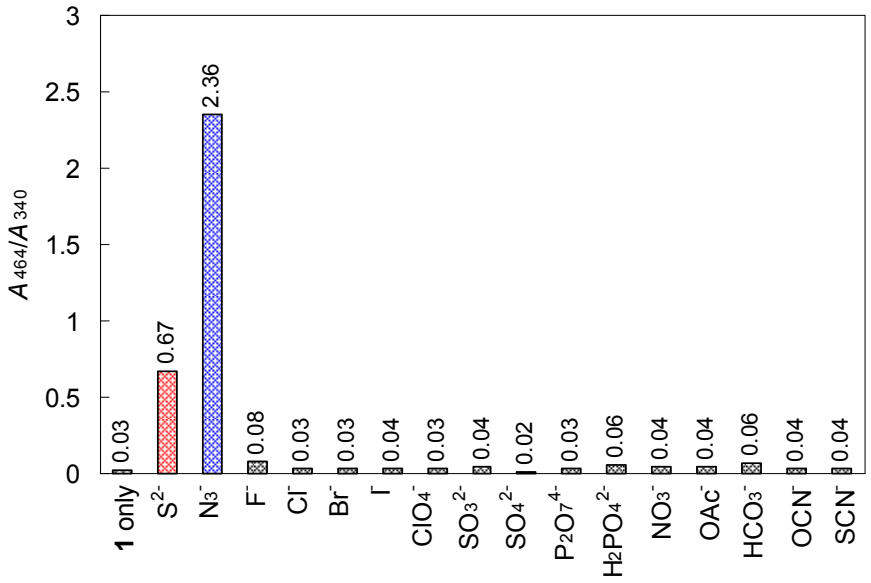


Fig. S3. UV–vis absorbance ratio at 464 nm and 340 nm (A_{464}/A_{340}) of **1** in the presence of representative anions. [**1**] = 3.0 × 10^{-5} M, [A^{n-}] = 3.0 × 10^{-4} M. In a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v).

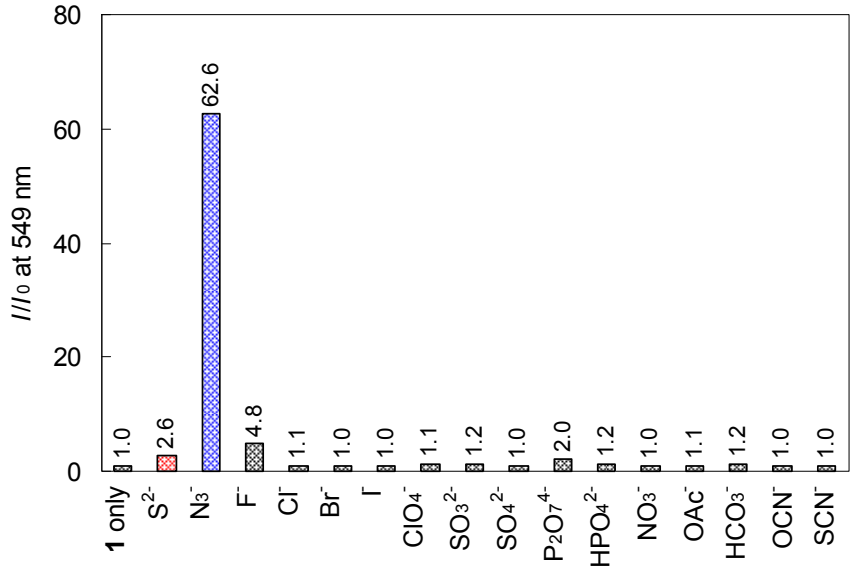


Fig. S4. Fluorescence intensity ratio (I/I_0) of **1** at 549 nm in the presence of representative anions. [**1**] = 1.5 × 10^{-5} M, [A^{n-}] = 1.5 × 10^{-4} M. We used a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v) for the measurements. λ_{ex} = 355 nm.

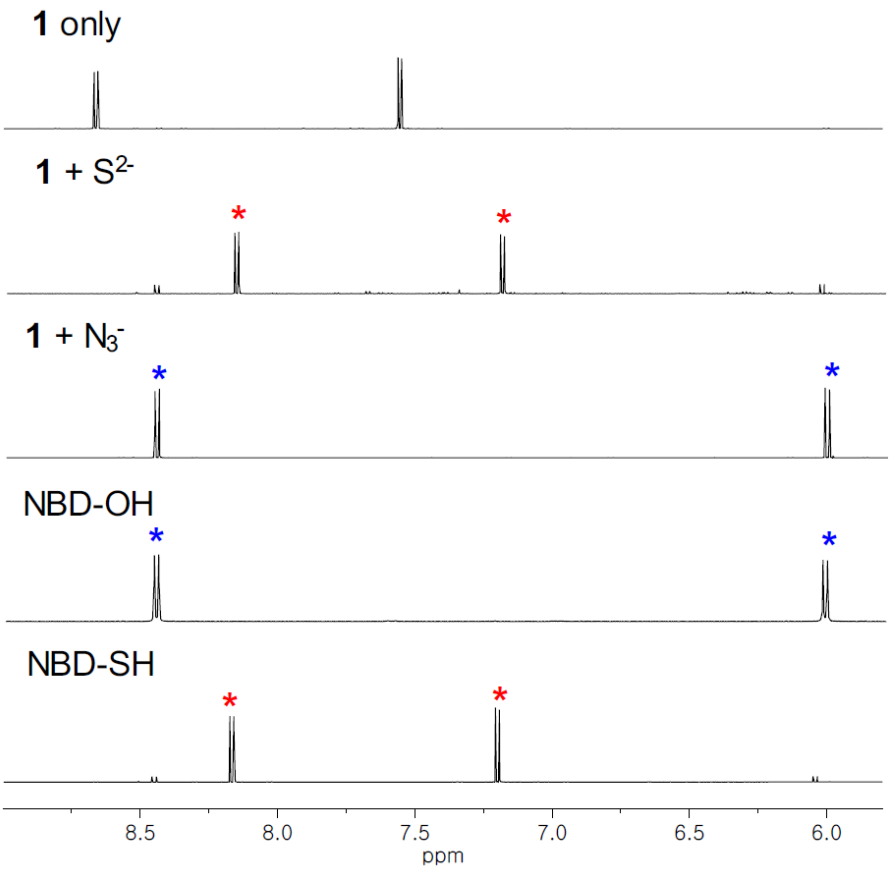


Fig. S5. Partial ^{1}H NMR spectra of **1**, NBD–OH **3**, NBD–SH, and **1** in the presence of S^{2-} and N_3^- ions. [**1**] = [NBD–OH] = [NBD–SH] = 5.0 mM in CD_3OD. Spectra (**1**+N_3^-) and (**1**+S^{2-}) were measured after column chromatography of the signaling products obtained from the reaction of **1** with two equiv of N_3^- and S^{2-} ions.

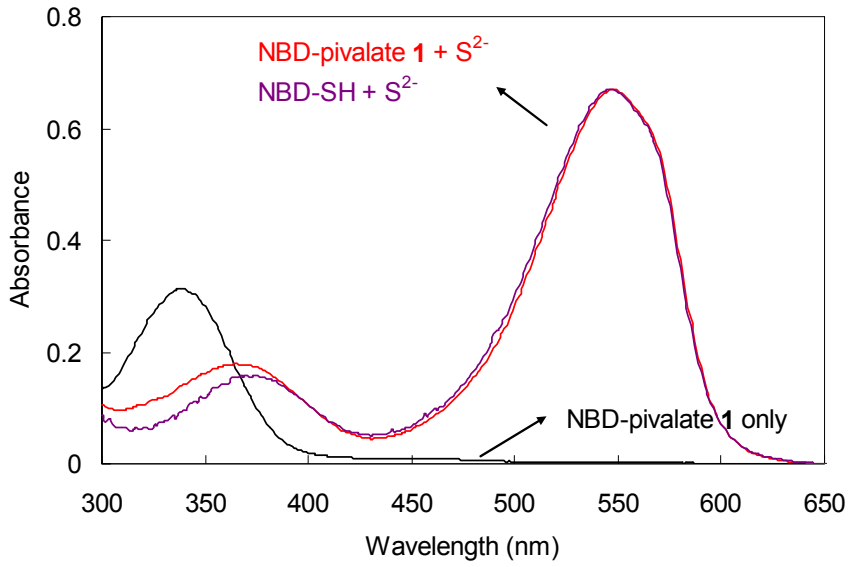


Fig. S6. UV–vis spectra of NBD–pivalate **1** and NBD–SH in the presence of sulfide ions. [**1**] = [NBD–SH] = 3.0×10^{-5} M, [S^{2-}] = 3.0×10^{-4} M. We used a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v) for the measurements.

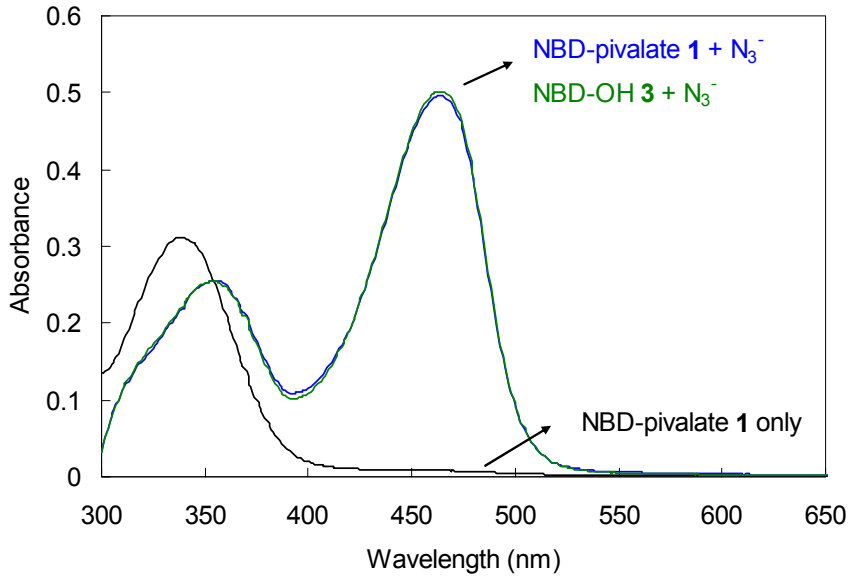
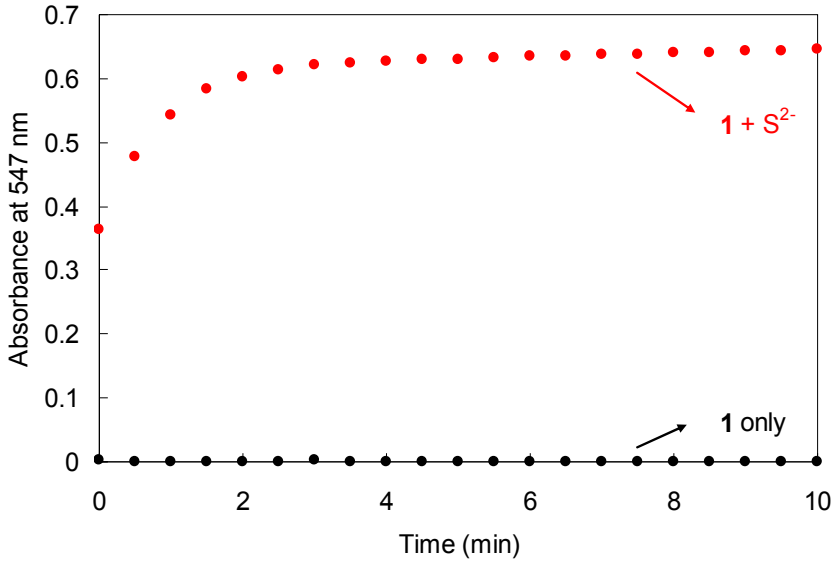


Fig. S7. UV–vis spectra of NBD–pivalate **1** and NBD–OH **3** in the presence of azide ions. [**1**] = [**3**] = 3.0×10^{-5} M, [N_3^-] = 3.0×10^{-4} M. We used a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v) for the measurements.

(a) Time-dependent UV–vis signaling of S^{2-} ions by **1**.



(b) Time-dependent UV–vis signaling of N_3^- ions by **1**.

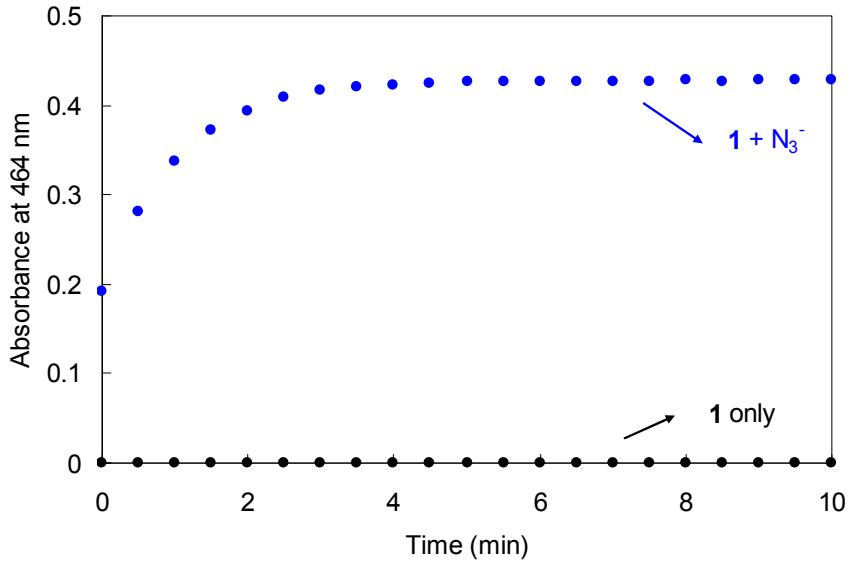
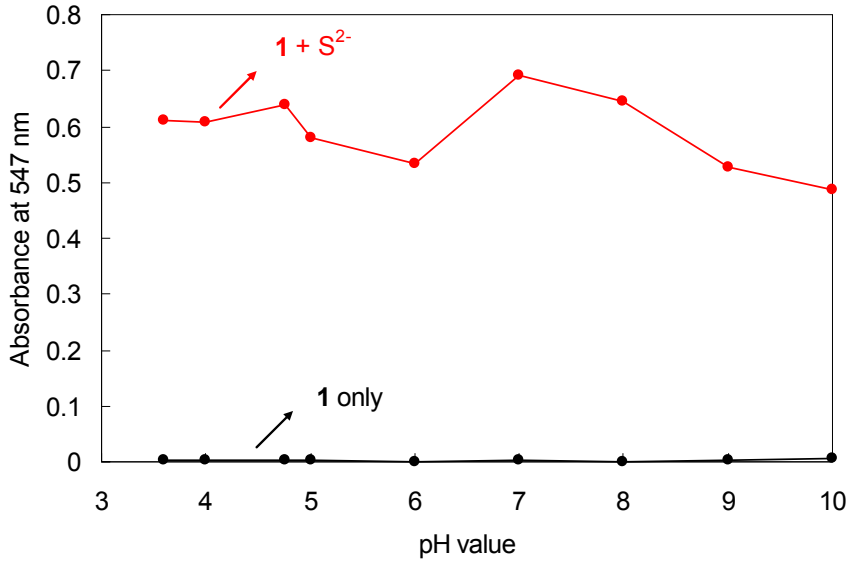


Fig. S8. Time-dependent UV–vis signaling of (a) S^{2-} and (b) N_3^- ions by **1**. [**1**] = 3.0 × 10^{-5} M, [S^{2-}] = [N_3^-] = 3.0 × 10^{-4} M. We used a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v) for the measurements.

(a) pH-dependency of S^{2-} signaling by **1**.



(b) pH-dependency of N_3^- signaling by **1**.

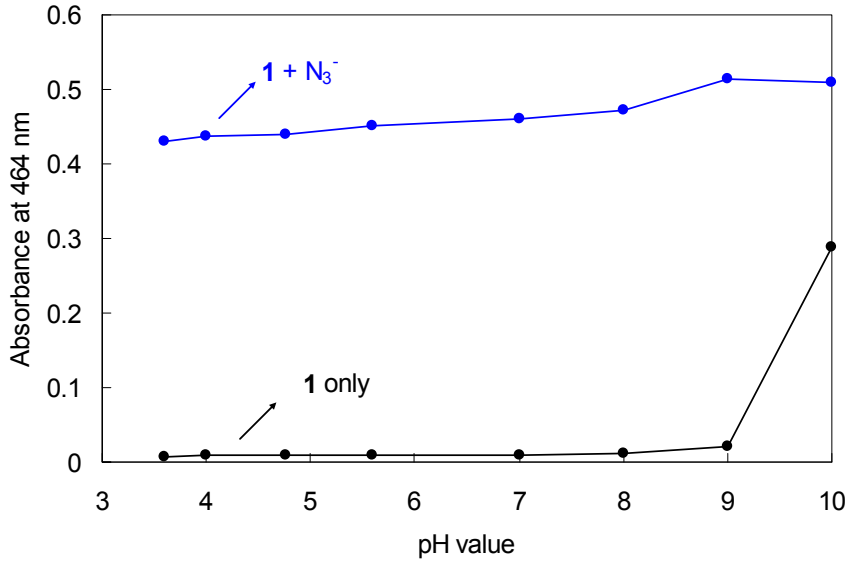


Fig. S9. pH-dependency of (a) S^{2-} and (b) N_3^- signaling by **1**. [**1**] = 3.0×10^{-5} M, [S^{2-}] = [N_3^-] = 3.0×10^{-4} M. We used a mixture of CH_3CN and buffer solution (20 mM), (1:1, v/v) for the measurements. Solution pH was adjusted by buffer solutions (pH 3.6 ~ 6.0: acetate buffer, pH 7.1: HEPES buffer, pH 8.0 ~ 9.0: tris buffer, pH 10.0: borate buffer).

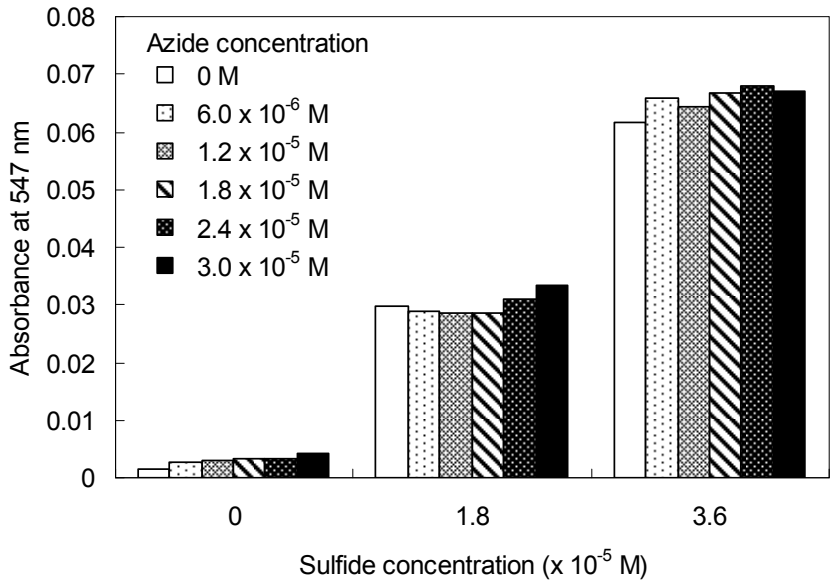


Fig. S10. UV–vis signaling of sulfide ions by **1** in the presence of varying concentration of azide ions. [**1**] = 3.0×10^{-5} M, [N_3^-] = from 0 to 3.0×10^{-5} M, [S^{2-}] = from 0 to 3.6×10^{-5} M. We used a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v) for the measurements.

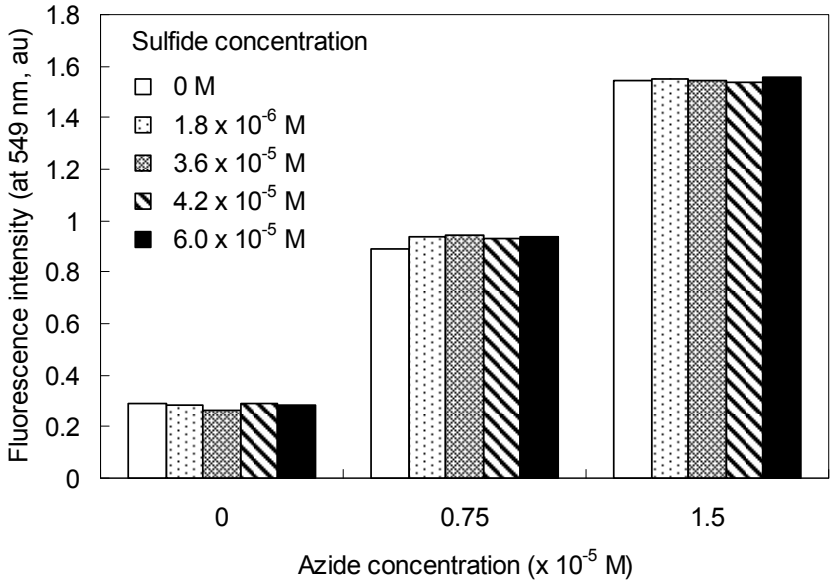


Fig. S11. Changes in fluorescence intensity (I_{549}) in the signaling of azide ions by **1** in the presence of varying concentration of sulfide ions. [**1**] = 1.5×10^{-5} M, [S^{2-}] = from 0 to 6.0×10^{-5} M, [N_3^-] = from 0 to 1.5×10^{-5} M. We used a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v) for the measurements. λ_{ex} = 355 nm.

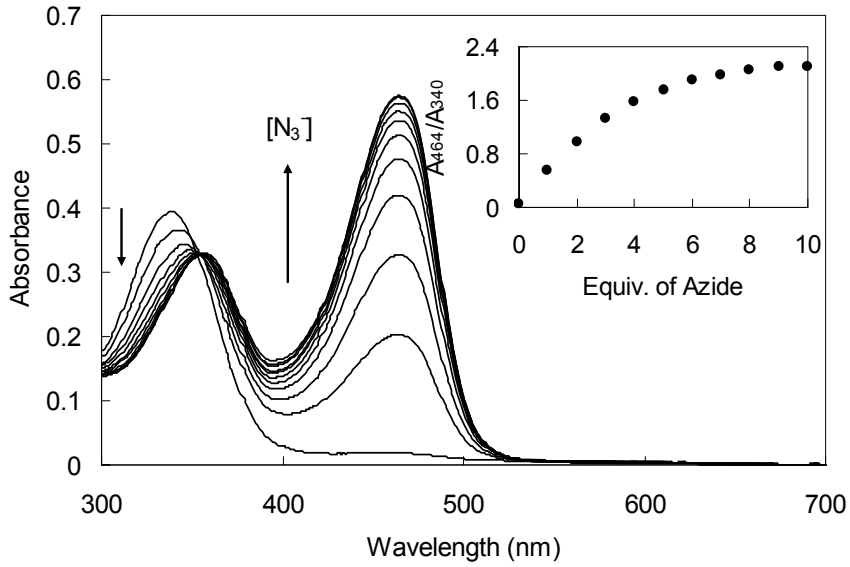


Fig. S12. Concentration-dependent UV–vis signaling of azide ions by **1**. [**1**] = 3.0×10^{-5} M, [N_3^-] = from 0 to 3.0×10^{-4} M. We used a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v) for the measurements.

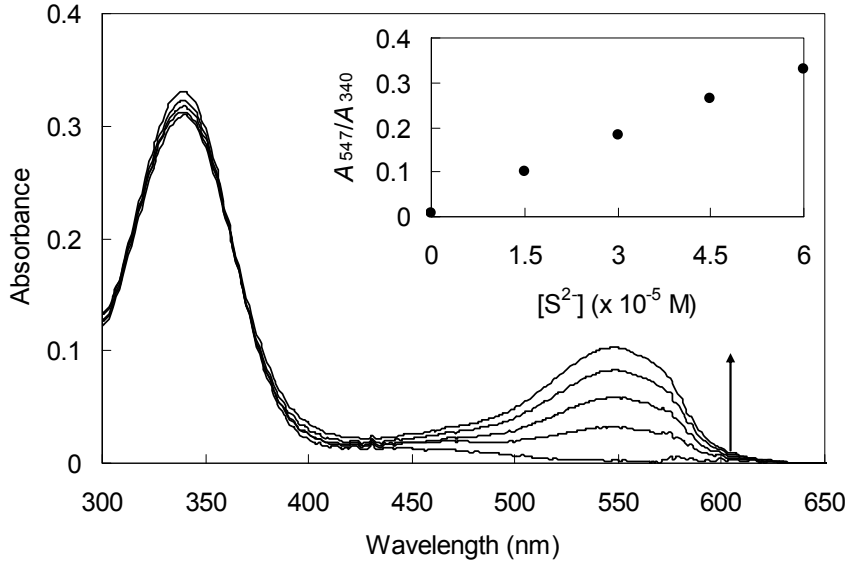


Fig. S13. Concentration-dependent UV–vis signaling of sulfide ions by **1** in simulated wastewater samples. [**1**] = 3.0×10^{-5} M, [S^{2-}] = from 0 to 6.0×10^{-5} M in a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v).

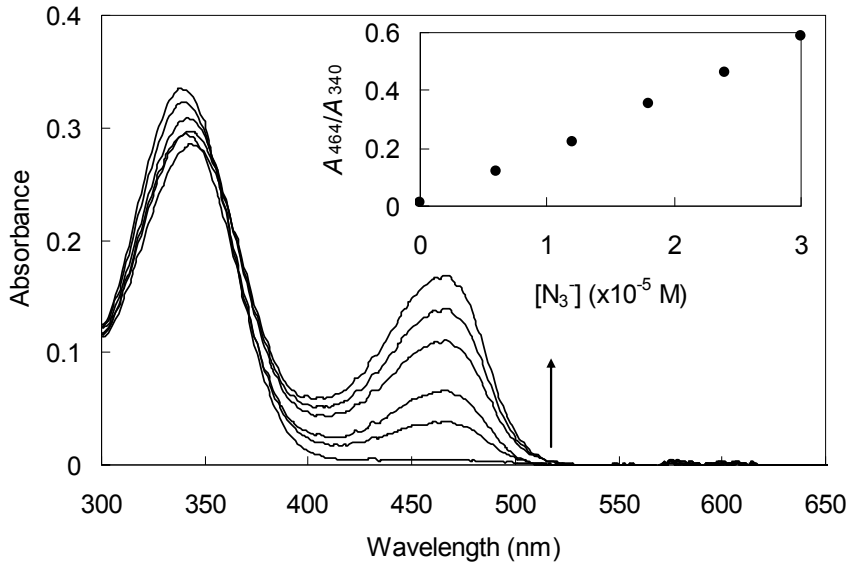


Fig. S14. Concentration-dependent UV–vis signaling of azide ions by **1** in simulated wastewater samples. [**1**] = 3.0×10^{-5} M, [N_3^-] = from 0 to 3.0×10^{-5} M in a mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM), (1:1, v/v).

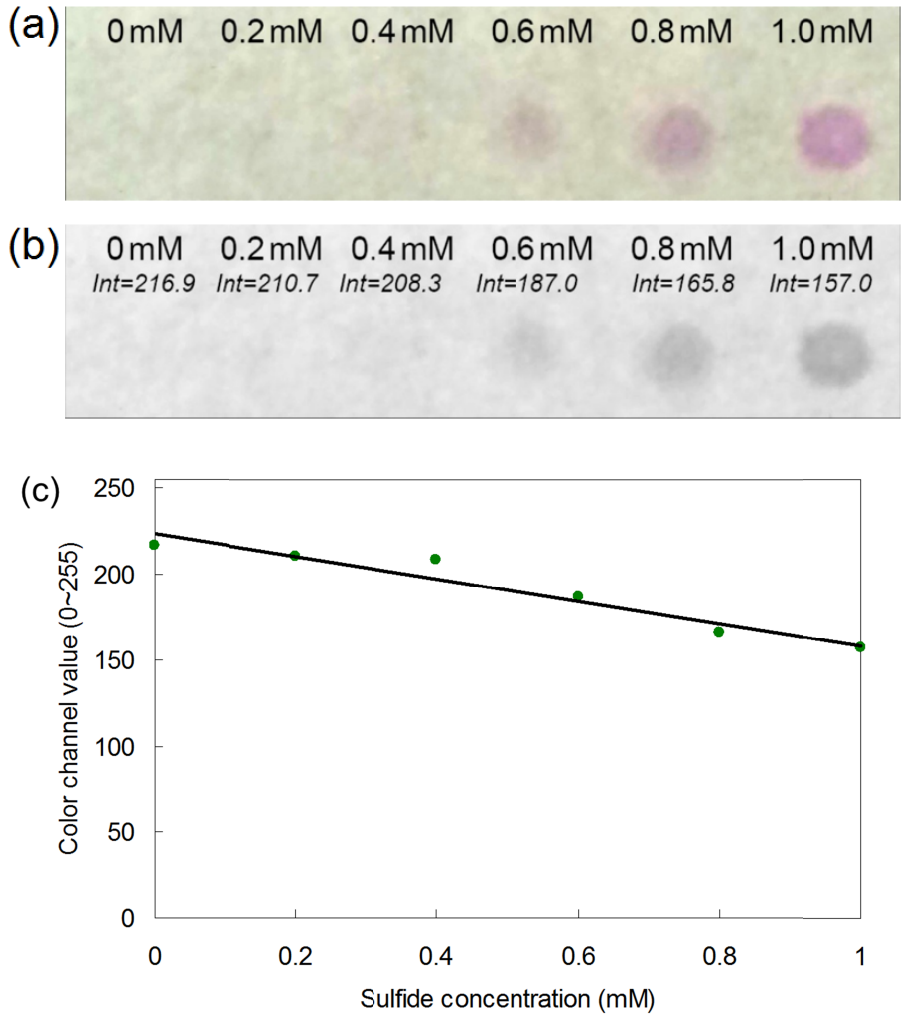
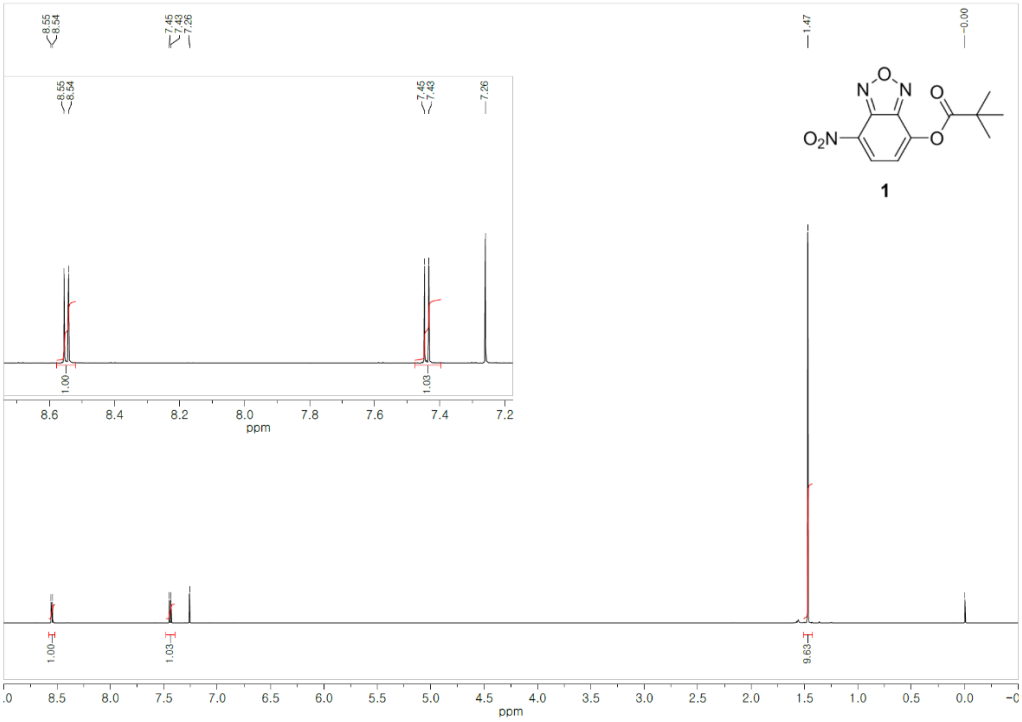


Fig. S15. Pictures of changes in (a) original, (b) green channel of **1** in the presence of varying amounts of sulfide ions, and (c) changes in green channel intensity of **1** as a function of sulfide ions. Intensities of green channel were obtained by recording color channel values (0–255) of image in Adobe Photoshop. $[S^{2-}]$ = from 0 to 1.0×10^{-3} M in distilled water.

(a) ^{1}H NMR spectrum of **1** in $CDCl_3$.



(b) ^{13}C NMR spectrum of **1** in $CDCl_3$.

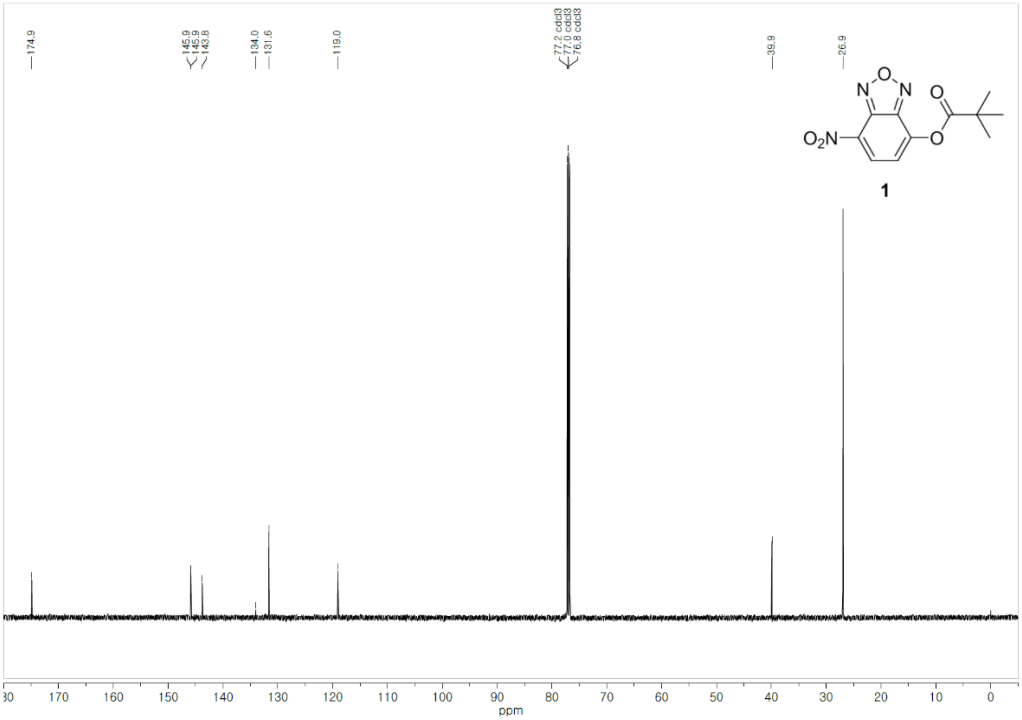
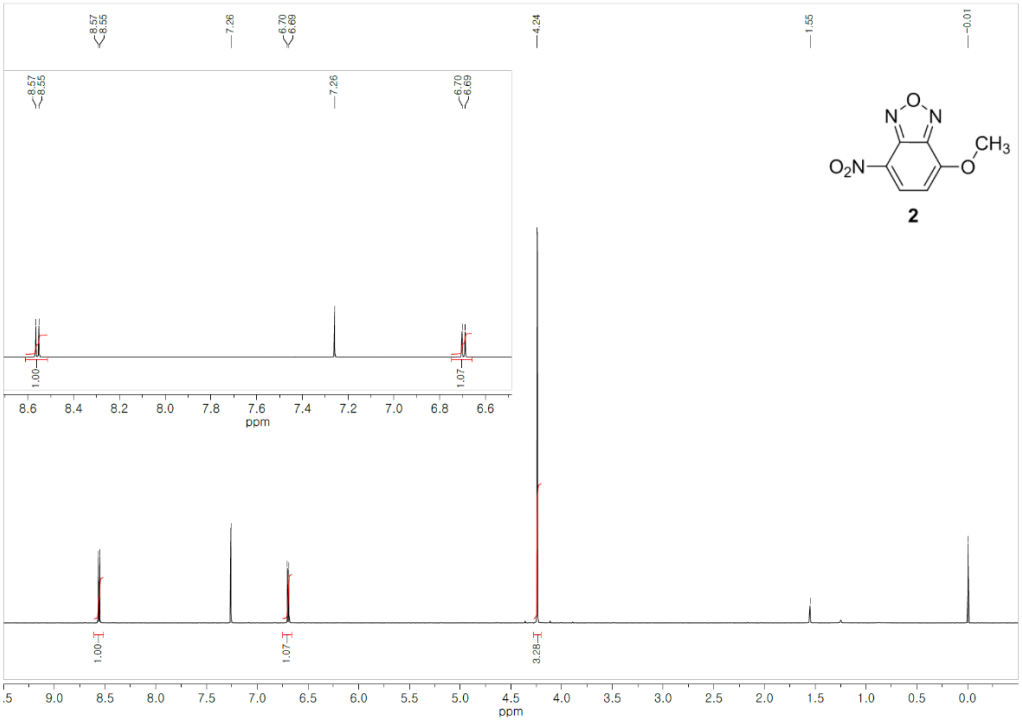


Fig. S16. ^{1}H NMR and ^{13}C NMR spectra of NBD–pivalate **1** in $CDCl_3$.

(a) ^{1}H NMR spectrum of **2** in $CDCl_3$.



(b) ^{13}C NMR spectrum of **2** in $CDCl_3$.

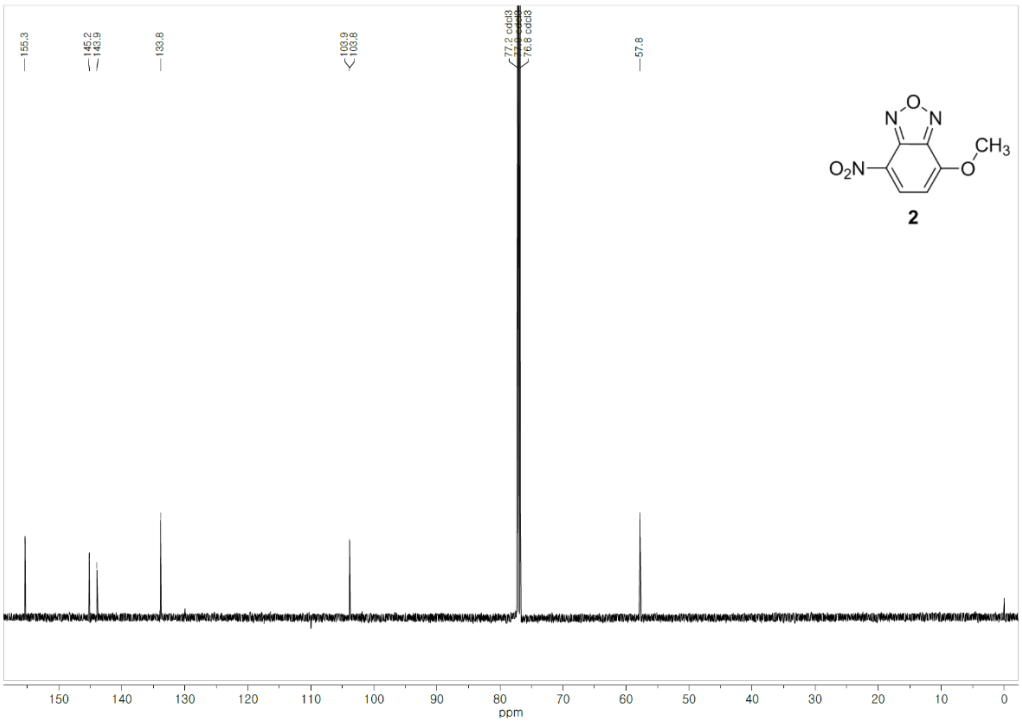
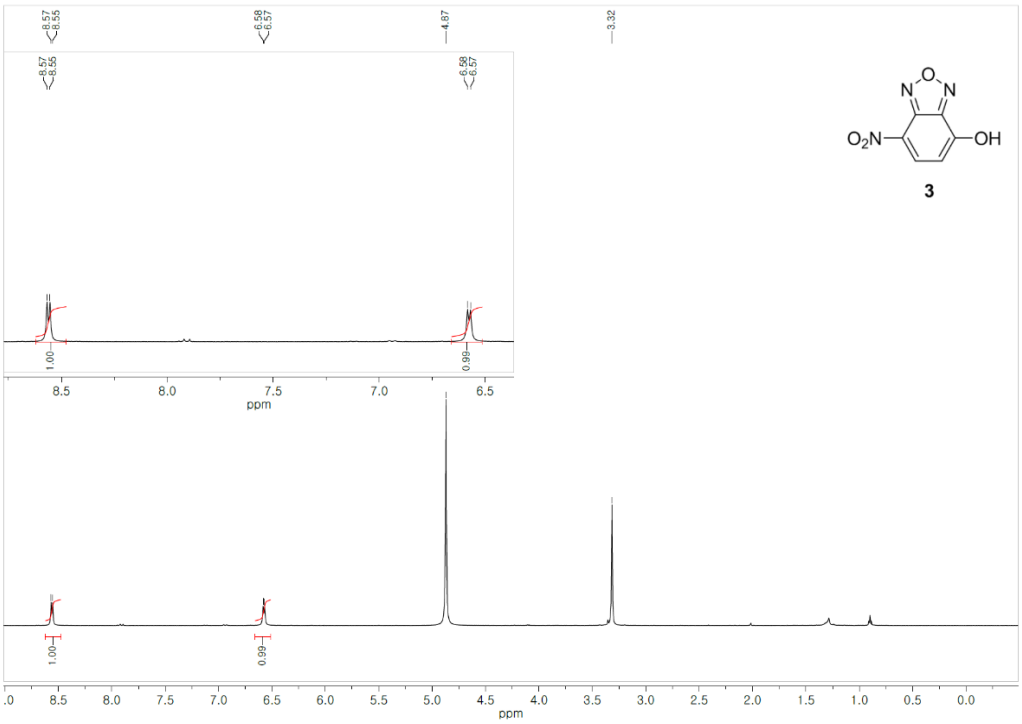


Fig. S17. ^{1}H NMR and ^{13}C NMR spectra of NBD–OCH_3 **2** in $CDCl_3$.

(a) ^{1}H NMR spectrum of **3** in CD_3OD.



(b) ^{13}C NMR spectrum of **3** in CD_3OD.

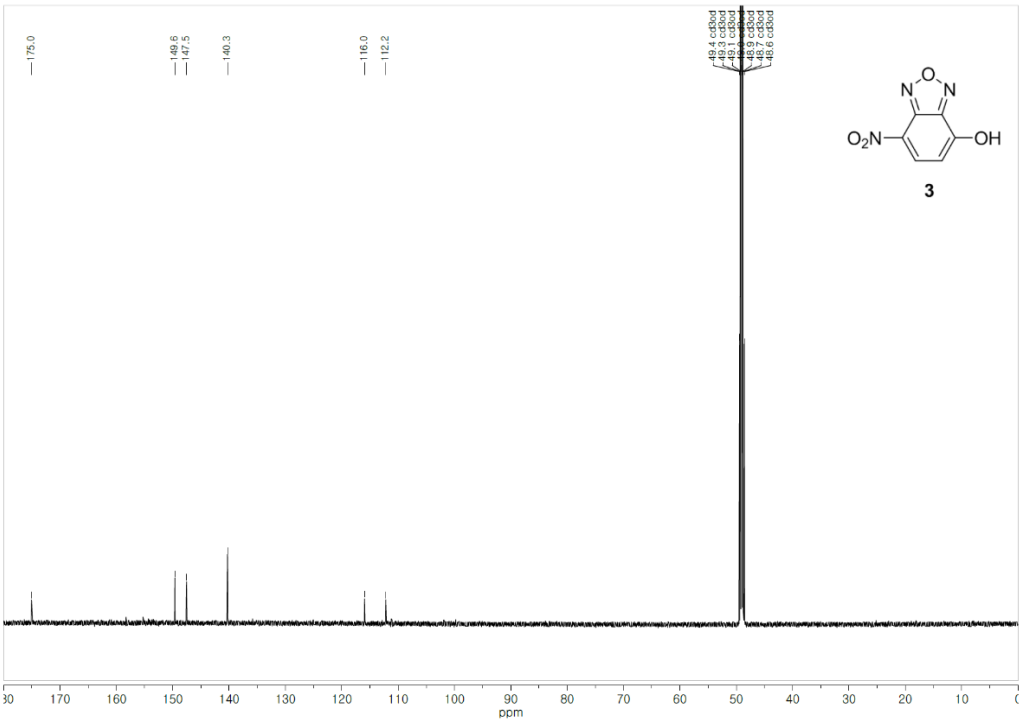
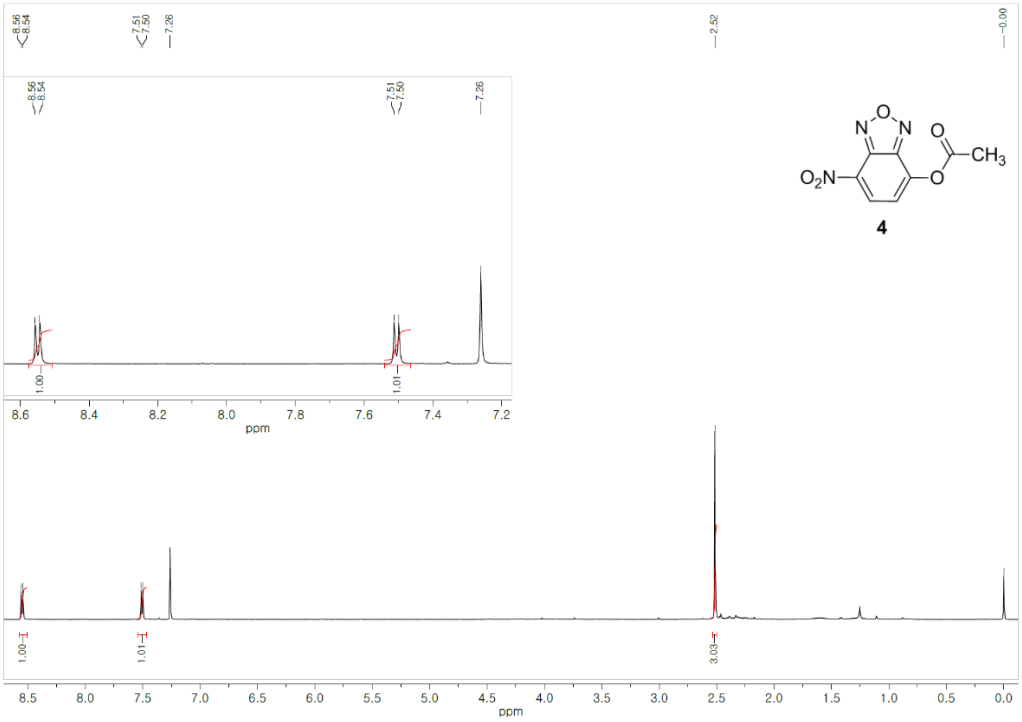


Fig. S18. ^{1}H NMR and ^{13}C NMR spectra of NBD–OH **3** in CD_3OD.

(a) ^{1}H NMR spectrum of **4** in $CDCl_3$.



(b) ^{13}C NMR spectrum of **4** in $CDCl_3$.

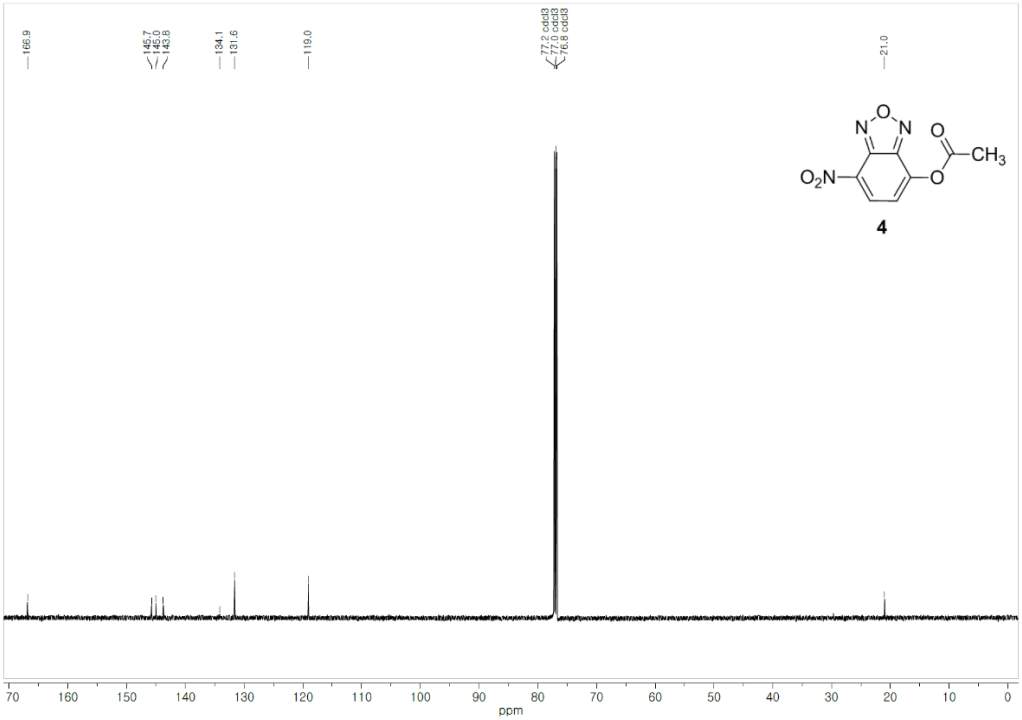
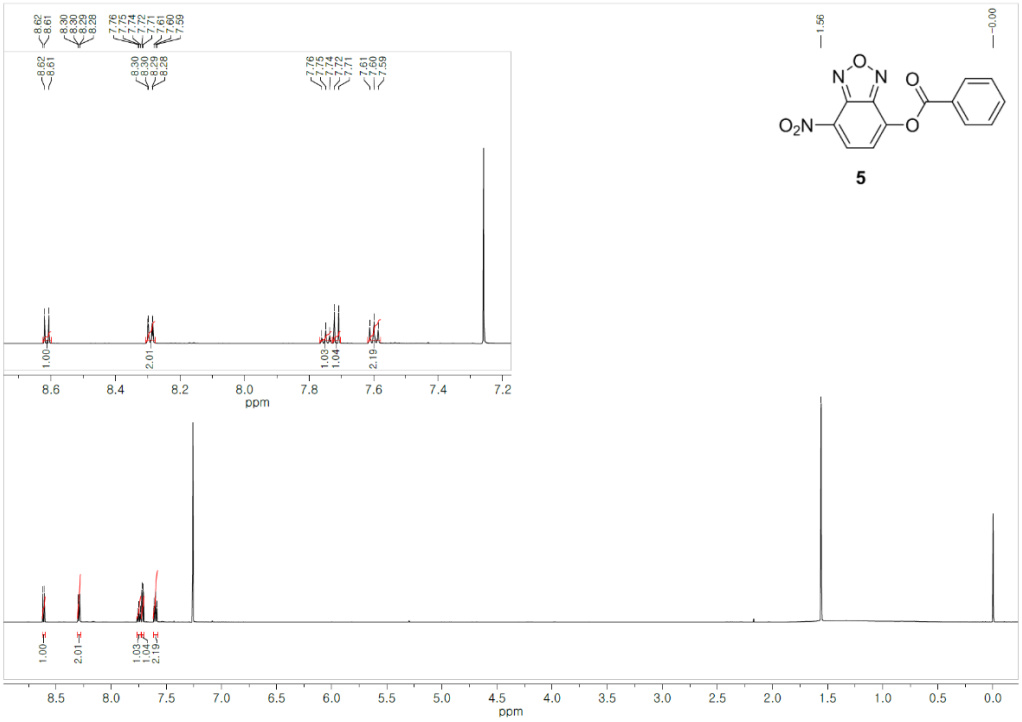


Fig. S19. ^{1}H NMR and ^{13}C NMR spectra of NBD–acetate **4** in $CDCl_3$.

(a) ^{1}H NMR spectrum of **5** in $CDCl_3$.



(b) ^{13}C NMR spectrum of **5** in $CDCl_3$.

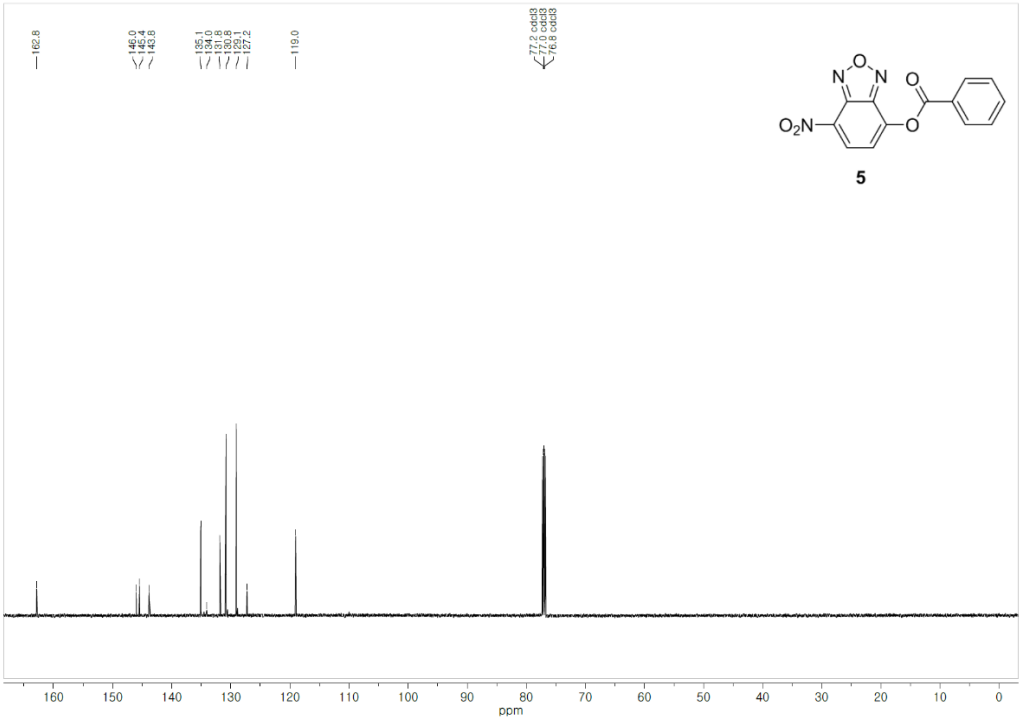


Fig. S20. ^{1}H NMR and ^{13}C NMR spectra of NBD–benzoate **5** in $CDCl_3$.