Electronic Supplementary Information (ESI)

Direct assay of butyrylcholinesterase activity using a fluorescent substrate

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Stability test of 1 in buffer condition

Fluorescence spectra of **1** (50 μM) was recorded under different pH conditions (pH = 7.0, 7.4, 8.0) using Tris buffer (20 mM) for 1 h to find the appropriate conditions for enzymatic assay.

In all experiments, the final sample volume was 1 mL and all measurements were performed in 1 cm quartz cells at 25 °C with excitation at 355 nm.

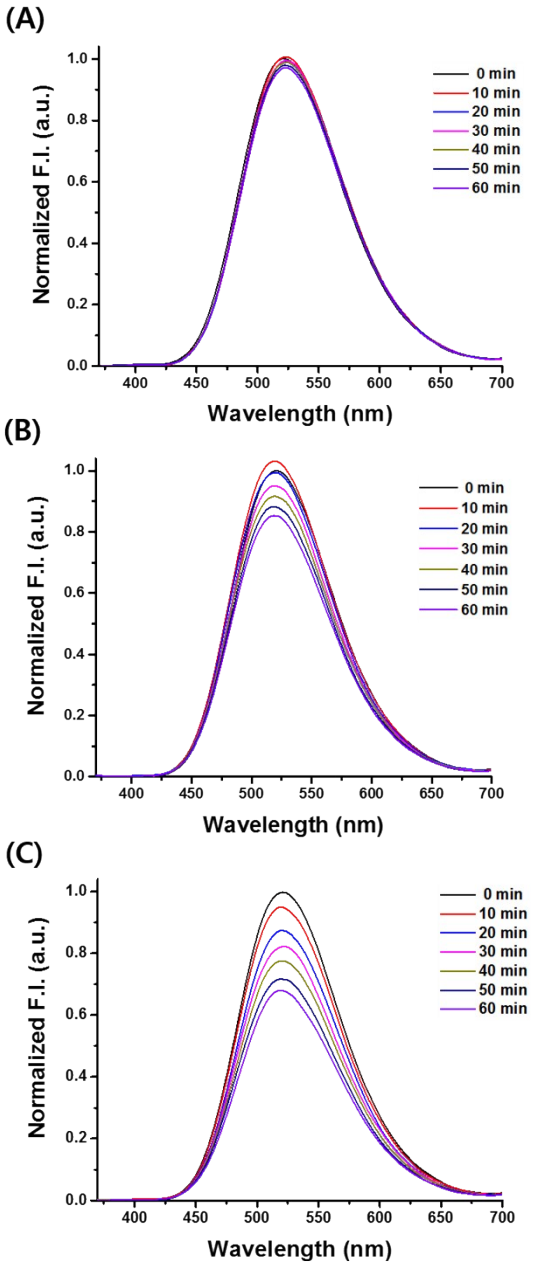


Figure S1. Stability test of **1** (50 μM, Tris buffer 20 mM) in different pH condition (A) pH 7.0, (B) pH 7.4, (C) pH 8.0. λ_{ex} = 355 nm.

Mechanism studies of BChE assay based on 1

BChE (2 U/mL) was then added to the sample containing **1** (100 μM) and Tris buffer (20 mM, pH 7.0) and the mixture was incubated for 90 min. By mass spectroscopy, the fragment formed by the enzymatic hydrolysis of **1** was confirmed.

A sample containing **1** (50 μM) and Tris buffer (20 mM, pH 7.0) in distilled H_2O and another sample containing **3** (50 μM) and Tris buffer (20 mM, pH 7.0) in 1% DMSO were prepared. Then, the fluorescence spectrum of each sample was recorded.

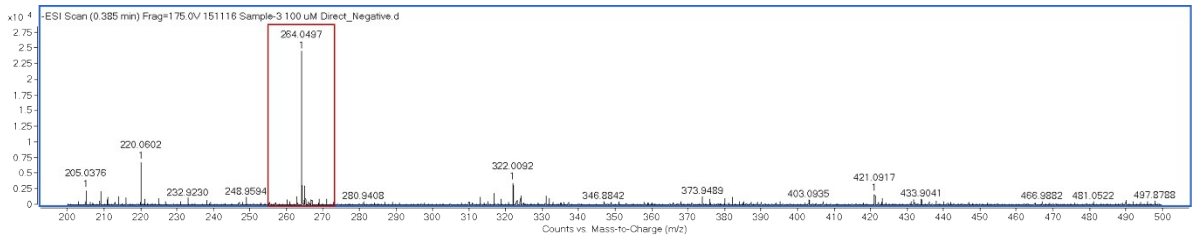


Figure S2. Mass spectrum of **1** (100 μM, Tris buffer pH 7.0 20 mM) containing BChE (2 U/mL) after incubation for 2 hours.

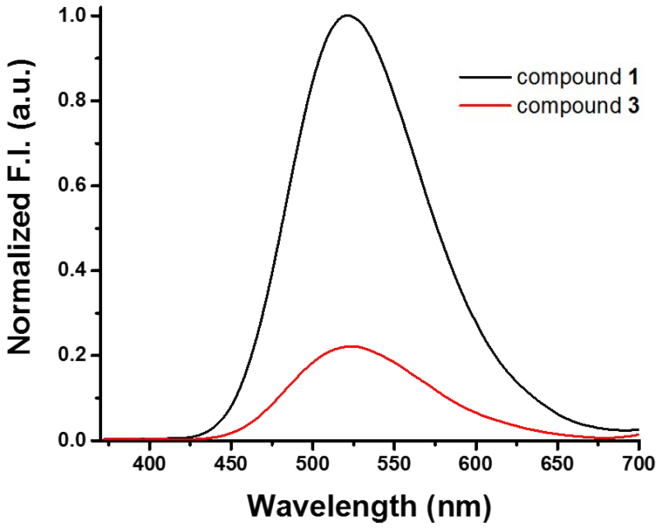


Figure S3. Comparison of fluorescence property of **1** (50 μM, Tris buffer pH 7.0 20 mM) and **3** (50 μM, Tris buffer pH 7.0 20 mM). λ_{ex} = 355 nm.

Selectivity test of 1 for BChE

BChE and other esterase were used; carboxylesterase from porcine liver (CES, EC 3.1.1.1), lipase from porcine pancreas (EC 3.1.1.3), and phospholipase C from *Clostridium perfringens* (PLC, EC 3.1.4.3). Each of esterase (2 U/mL) was added to the sample containing **1** (50 μM) and Tris buffer (20 mM, pH 7.0), and fluorescence spectra of each sample were recorded for 30 min.

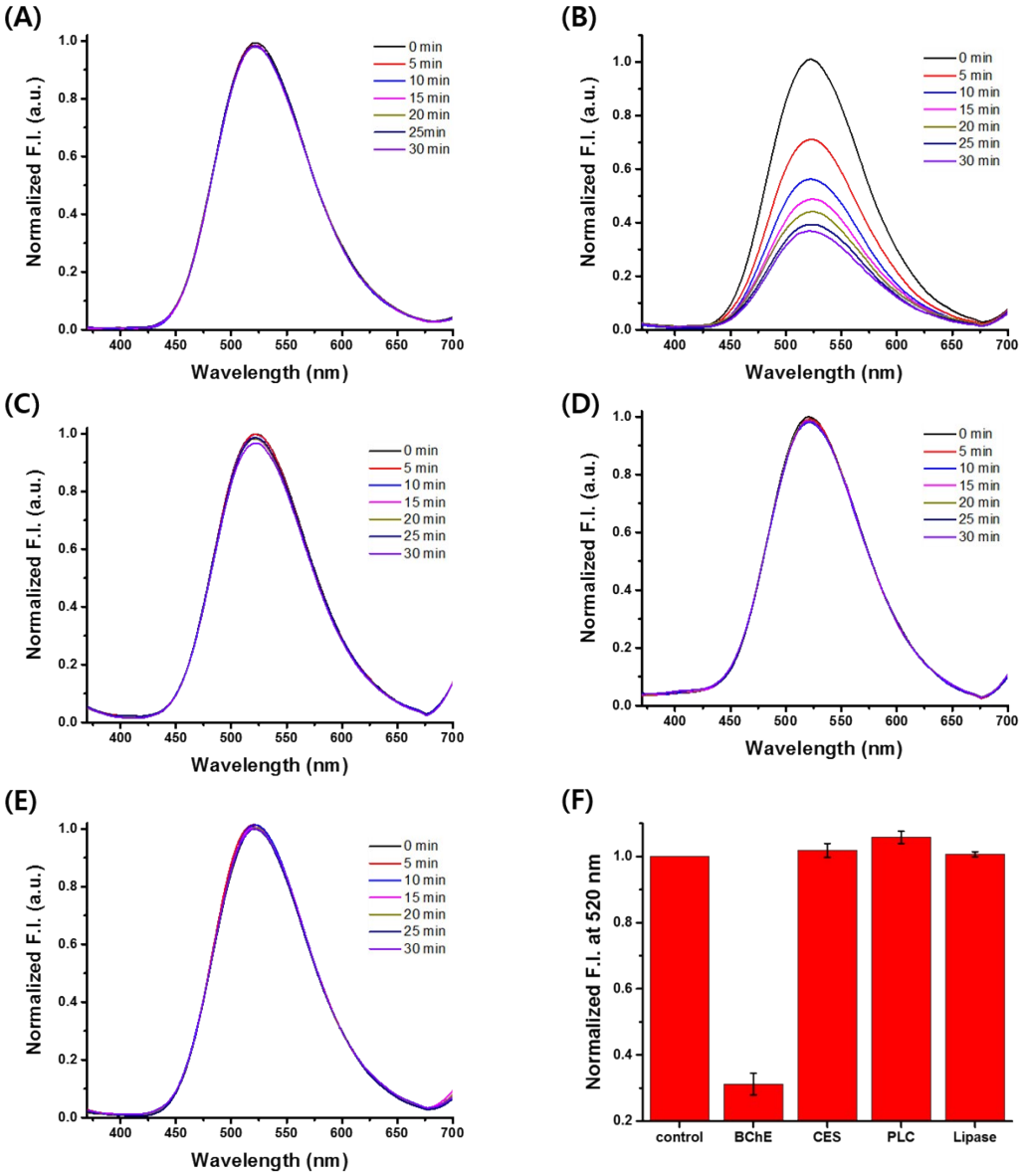
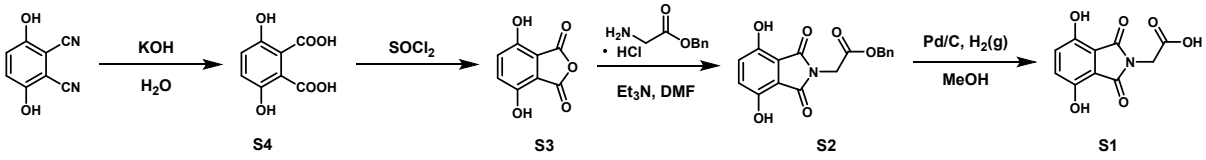


Figure S4. Fluorescence spectra of **1** (50 μM, Tris buffer 20 mM) with different esterase (2 U/mL), (A) without esterase, (B) BChE, (C) CES, (D) PLC, (E) lipase, (F) Normalized fluorescence intensities at 520 nm of **1** *versus* different esterase (2 U/mL) after 30 min incubation. λ_{ex} = 355 nm.

Model study of 3,6-dihydroxy phthalimide derivatives

In order to confirm the substitution effect from dimethoxy phthalimide part to dihydroxy phthalimide in compound **1**, we synthesized 3,6-dihydroxy phthalimide derivative (**S1**, **S2**) for comparison study (Scheme S1).



Scheme S1. Synthesis of 3,6-dihydroxy phthalimide derivatives (**S1**, **S2**) for model study.

1) Synthesis of 3,6-dihydroxyphthalic acid (**S4**) and 4,7-dihydroxyisobenzofuran-1,3-dione (**S3**).

S4 and **S3** was synthesized by following literature procedures. (*Phys. Chem. Chem. Phys.*, 2015, **17**, 30659-30669.)

2) Synthesis of benzyl 2-(4,7-dihydroxy-1,3-dioxoisoindolin-2-yl)acetate (**S2**).

Et_3N (0.74 mL, 5.25 mmol) and **S3** (0.90 g, 5.00 mmol) were added to a solution of Glycine benzylester hydrochloride (1.06 g, 5.25 mmol) in DMF (6 mL) at 0 °C. After stirring for 30 min, reaction mixture was heated at 70 °C for 14 h. The brown solution was cooled to r.t. and diluted with TDW (50 mL), and extracted with EtOAc (50 mL × 3). The organic phase was washed with TDW and brine and dried with anhydrous Na_2SO_4. The filtrate was then concentrated and residue as purified by column chromatography (silica gel, $CHCl_3$: MeOH = 10: 1, v/v) to obtain **S2** (0.43 g, 26 %) as a pale red powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.91 (br.s, 2H), 7.40-7.32 (m, 5H), 7.10 (s, 2H), 5.18 (s, 2H), 4.35 (s, 2H) ppm. ^{13}C NMR

(100 MHz, DMSO-d_6): δ 167.90, 165.59, 148.43, 135.58, 128.51, 128.25, 128.00, 126.32, 113.82, 66.57, 38.34 ppm.

3) Synthesis of 2-(4,7-dihydroxy-1,3-dioxoisoindolin-2-yl)acetic acid (**S1**).

A solution of **S2** (0.23 g, 0.7 mmol) in MeOH (15 mL) was hydrogenated at atmospheric pressure for 30 min at room temperature using 10% palladium-carbon (0.14 g) as a catalyst. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to obtain **S1** (0.14 g, 84 %) as a yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.31 (br.s, 2H), 7.10 (s, 2H), 4.16 (s, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.29, 165.78, 148.30, 126.18, 114.01, 38.38 ppm.

4) UV-Vis and Fluorescence spectra of **S1** and **S2** for various pH.

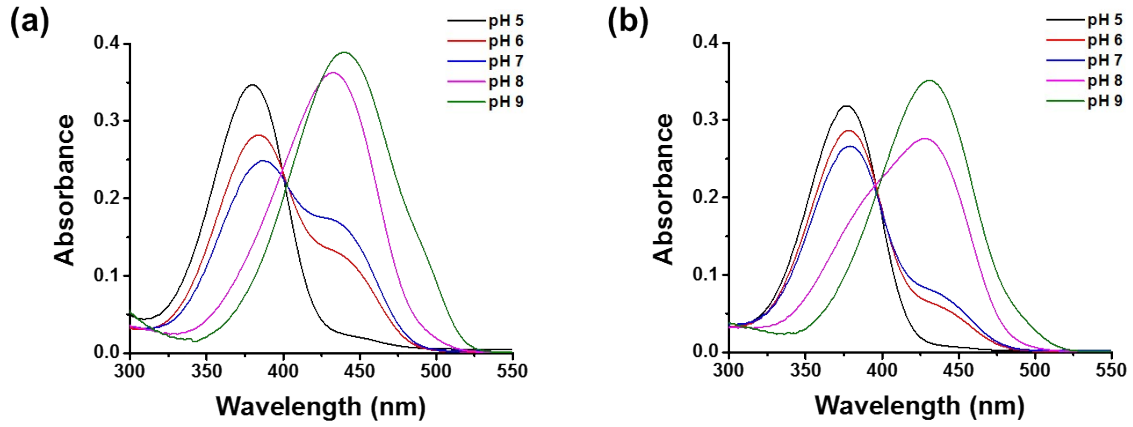


Figure S5. (a) UV-vis spectra of **S2** (50 μM) and (b) **S1** (50 μM) for various pH condition (20 mM, DMSO 5%). pH 5~6: citrate buffer, pH 7~9: Tris buffer.

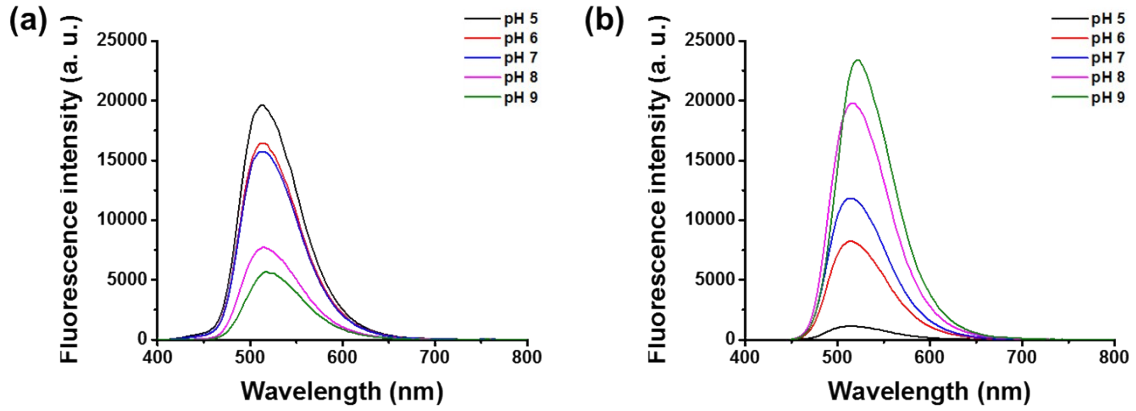


Figure S6. Fluorescence spectra of **S2** (50 μM) for various pH condition (20 mM, DMSO 5%). pH 5~6: citrate buffer, pH 7~9: Tris buffer. (a) λ_{ex} = 378 nm, (b) λ_{ex} = 436 nm

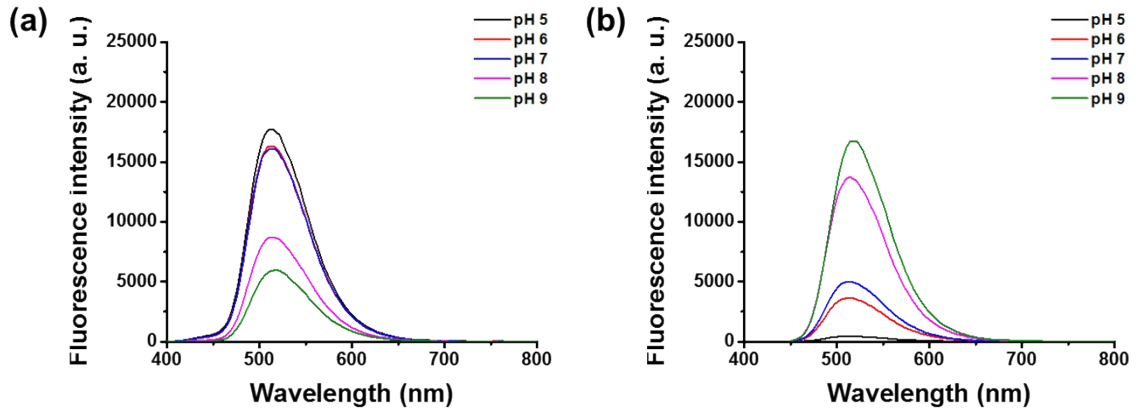


Figure S7. Fluorescence spectra of **S1** (50 μM) for various pH condition (20 mM, DMSO 5%). pH 5~6: citrate buffer, pH 7~9: Tris buffer. (a) λ_{ex} = 378 nm, (b) λ_{ex} = 436 nm

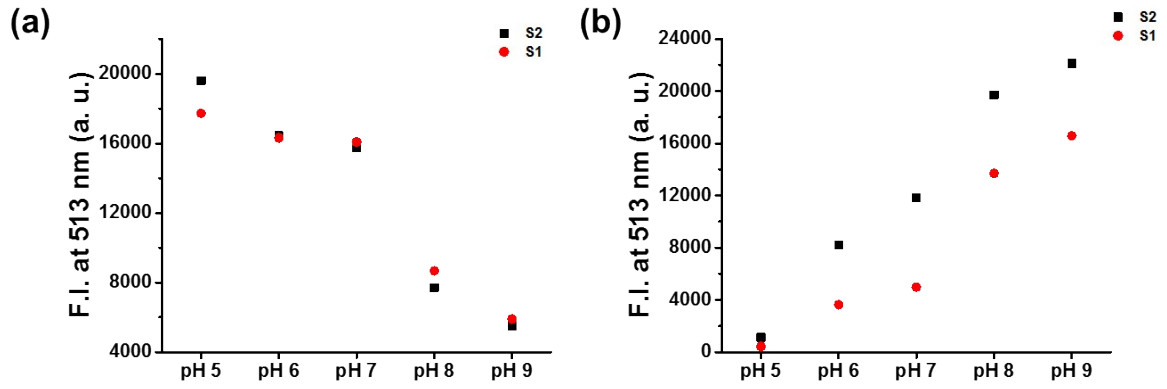
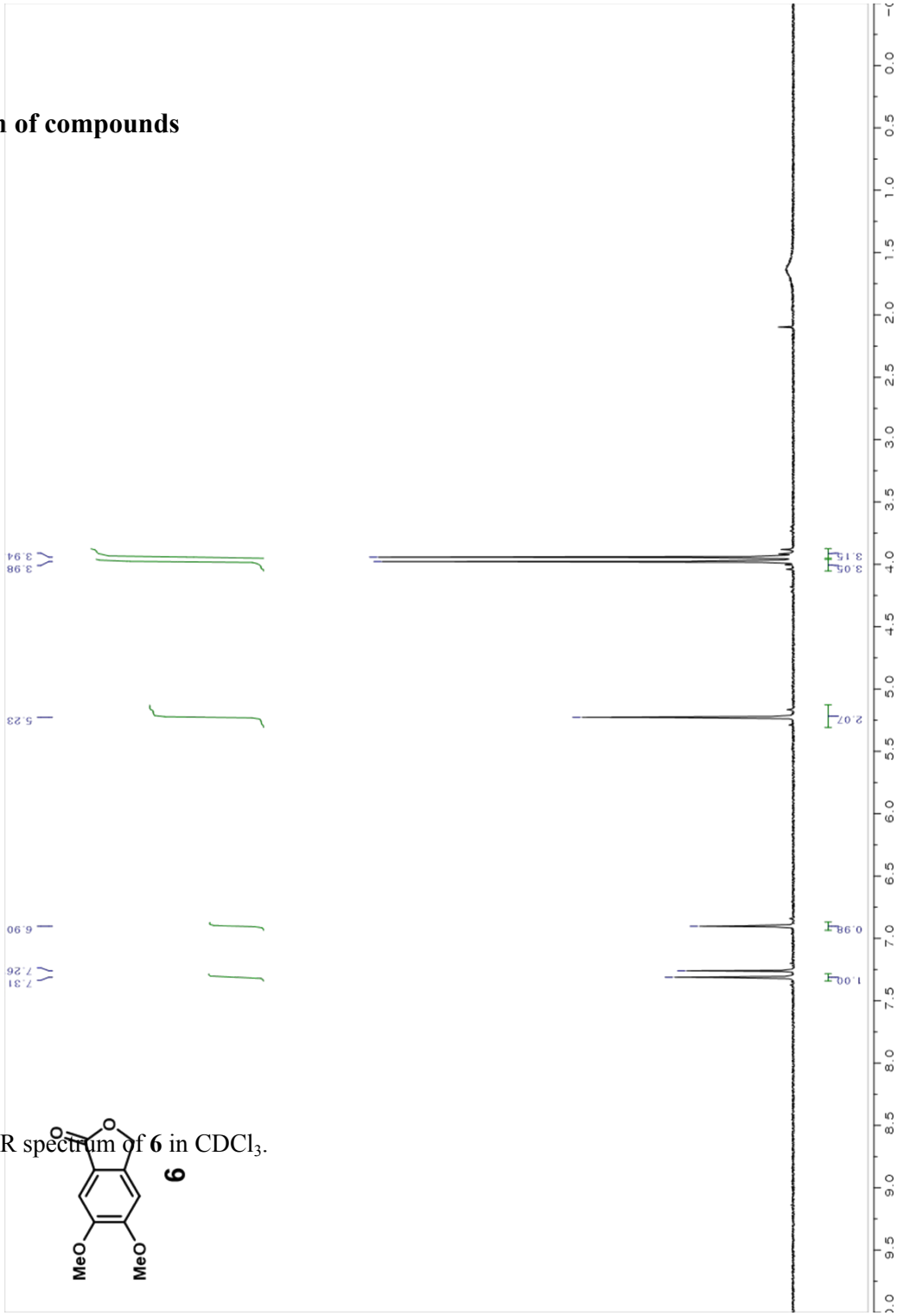


Figure S8. Plot of fluorescence intensity of **S2** (50 μM) and **S1** (50 μM) at 513 nm. pH 5~6: citrate buffer, pH 7~9: Tris buffer. (a) λ_{ex} = 378 nm, (b) λ_{ex} = 436 nm

Based on the UV-Vis spectra of **S1** and **S2**, we recorded fluorescence emission spectra of **S1** and **S2** for two difference excitation wavelength (378 nm, 436 nm). As shown Figure 4(a), in case of λ_{ex} = 378 nm, fluorescence intensity of **S1** and **S2** at 513 nm are almost same in various pH condition. And in case of λ_{ex} = 436 nm, **S2** (benzyl ester moiety) was showed more strong fluorescence intensity than **S1** (acid moiety). This experimental result is similar with that of dimethoxy moiety that fluorescence on-off phenomena.

Characterization of compounds



6

Figure S9. ^{1}H-NMR spectrum of **6** in $CDCl_3$.

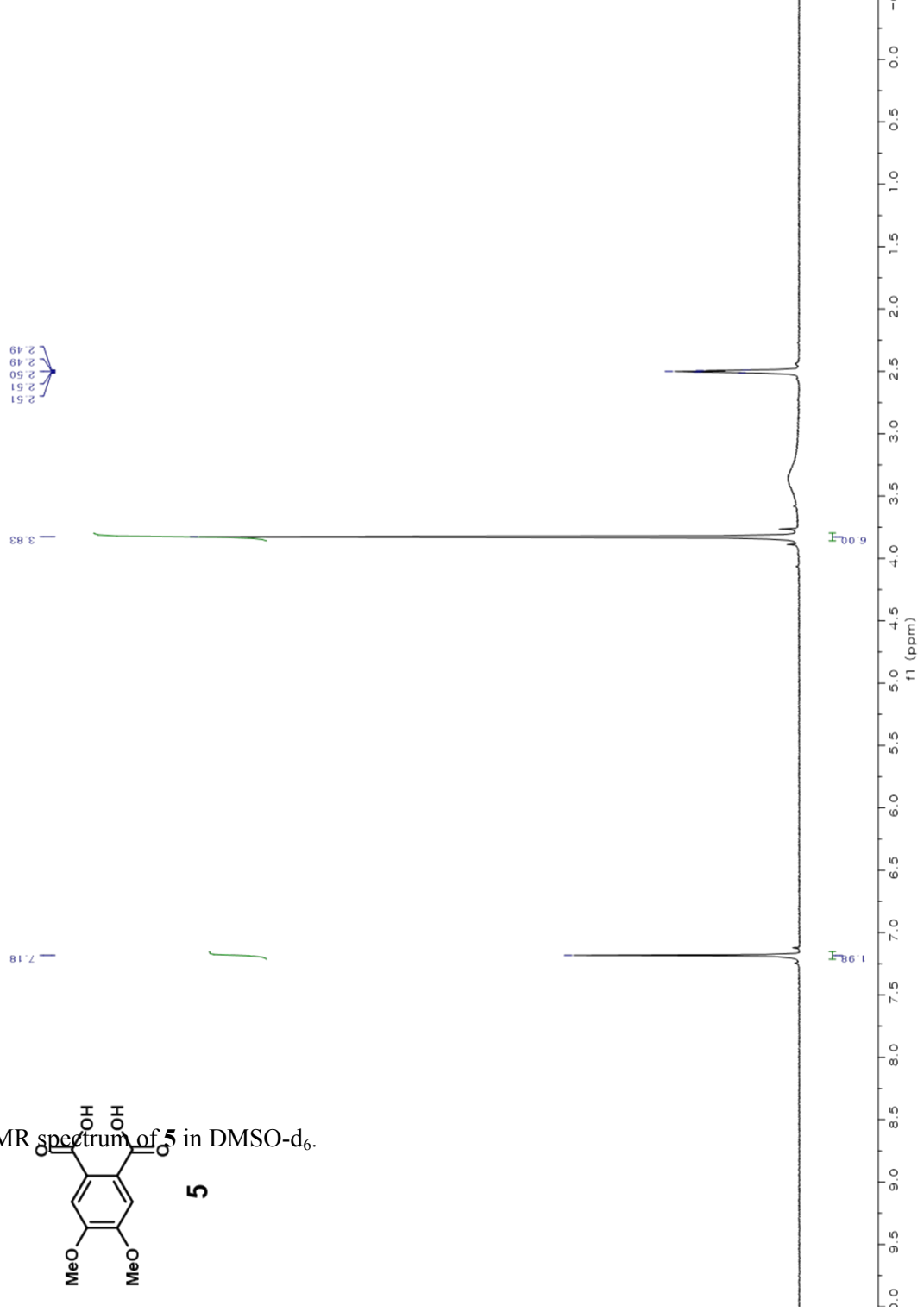


Figure S10. ^{1}H-NMR spectrum of **5** in DMSO-d_6.

5

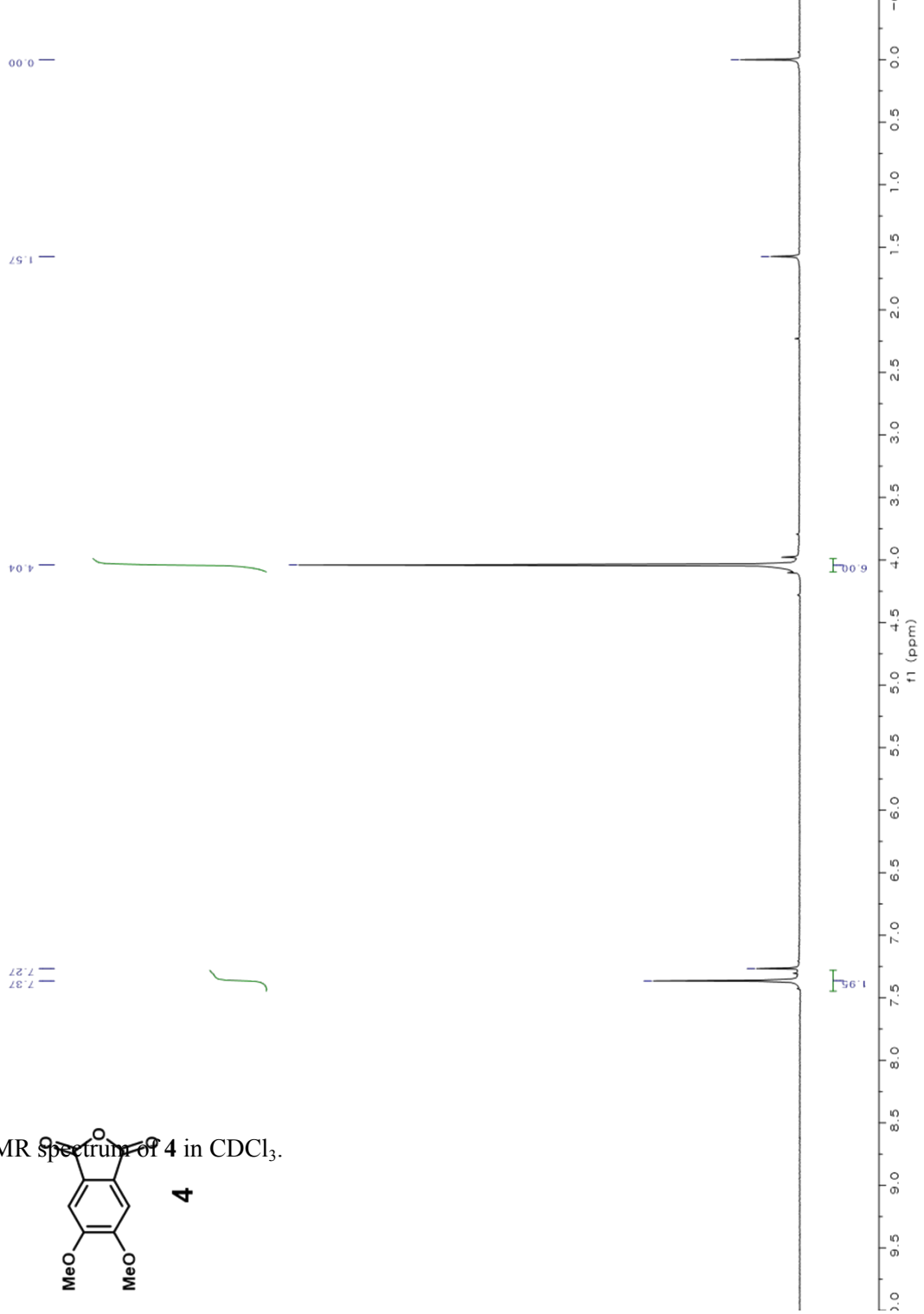
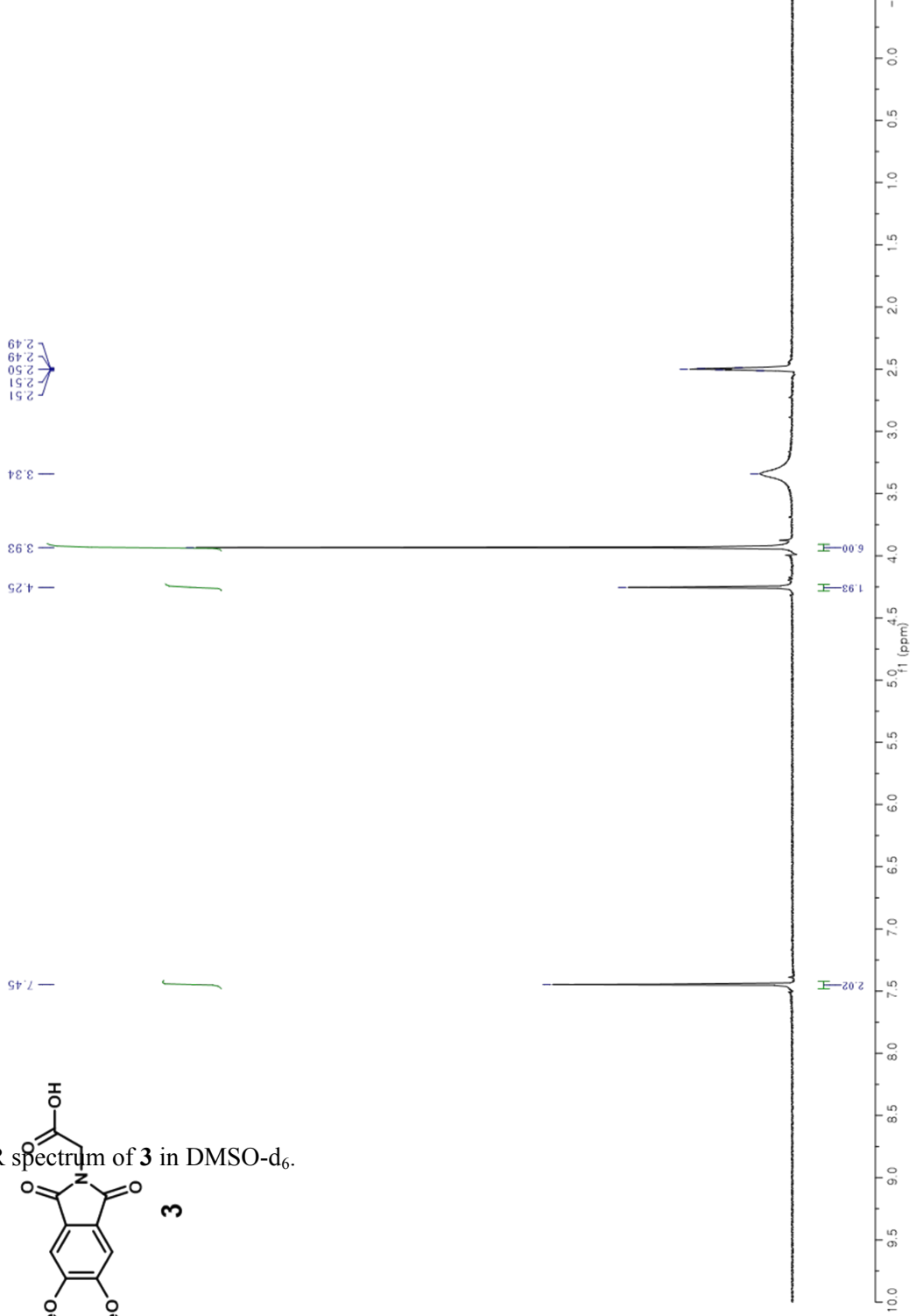


Figure S11. ^{1}H-NMR spectrum of **4** in $CDCl_3$.

4



3

Figure S12. ^{1}H-NMR spectrum of **3** in DMSO-d_6.

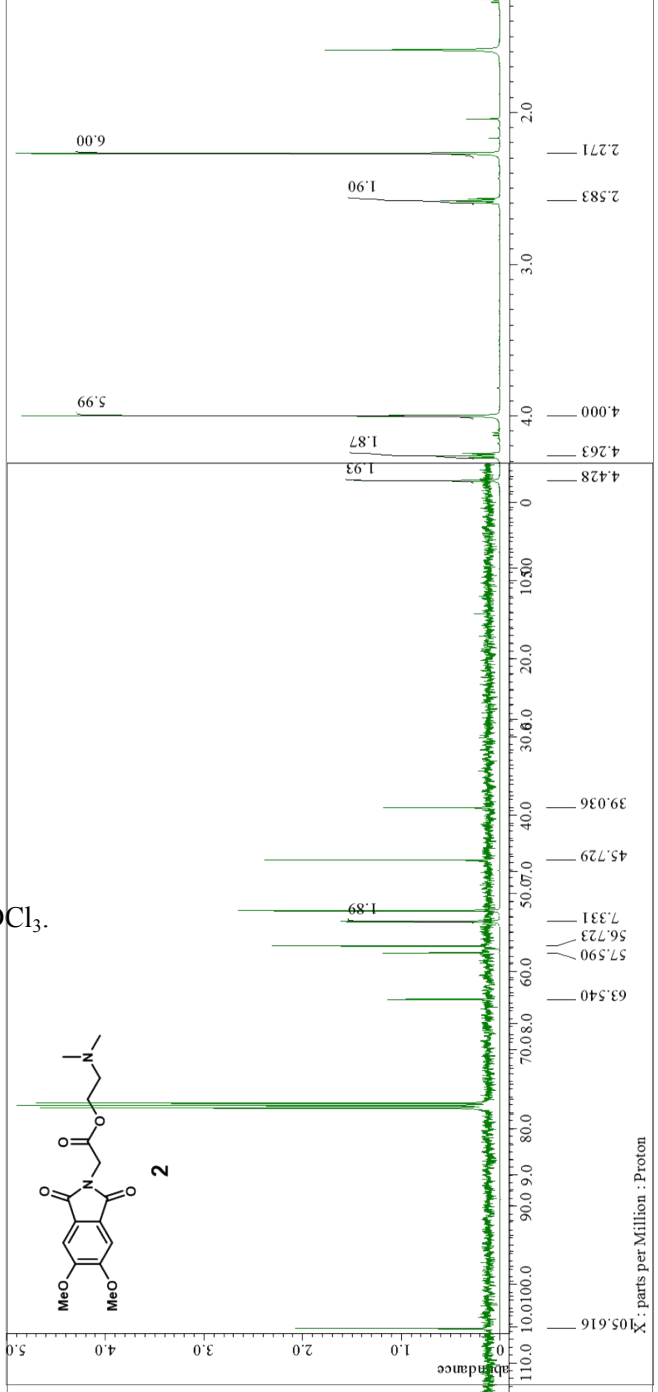


Figure S13. ^{1}H-NMR spectrum of **2** in $CDCl_3$.

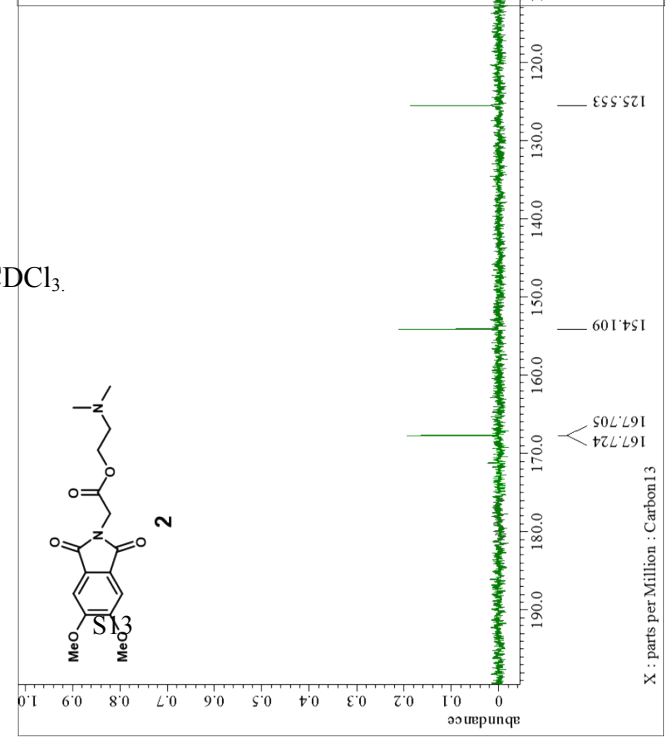


Figure S14. ^{13}C-NMR spectrum of **2** in $CDCl_3$.

Figure S15. ESI-Mass spectrum of **2**.

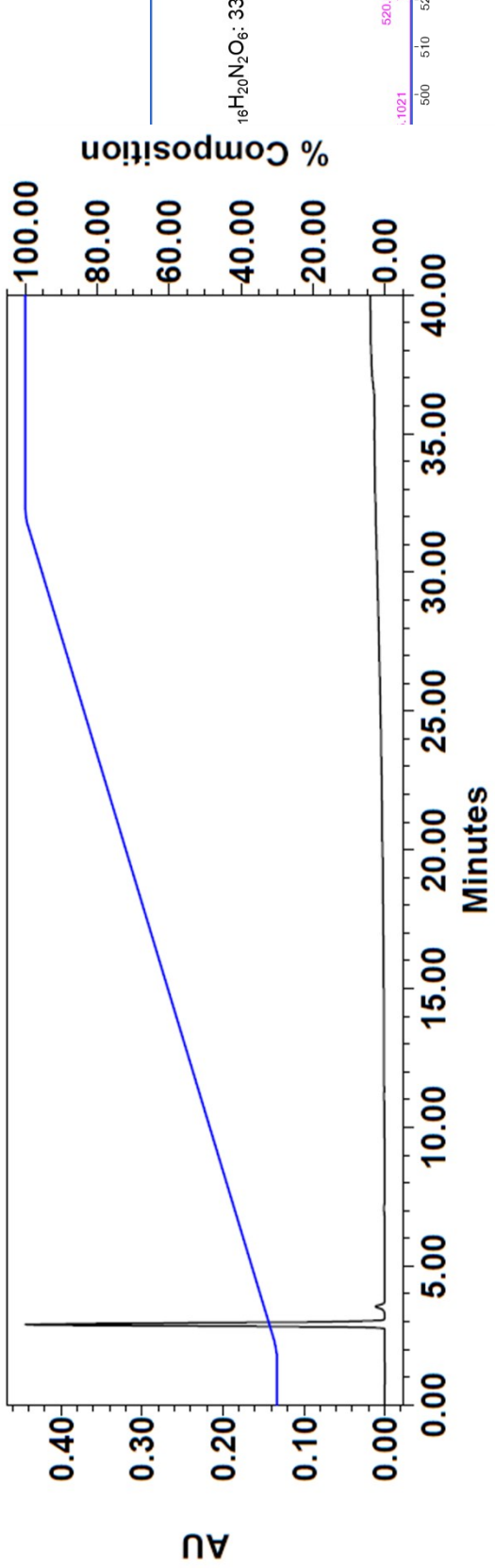


Figure S16. HPLC chromatogram of **2**.

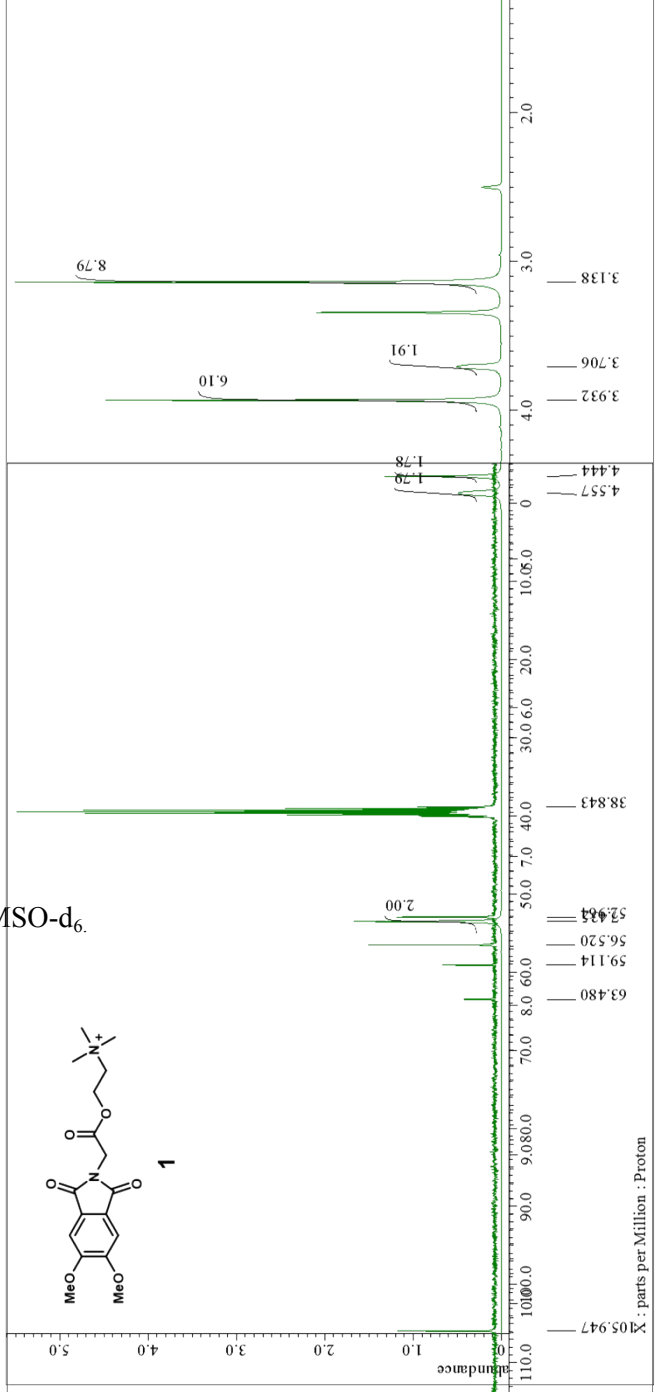


Figure S17. ^{1}H-NMR spectrum of **1** in DMSO-d_6.

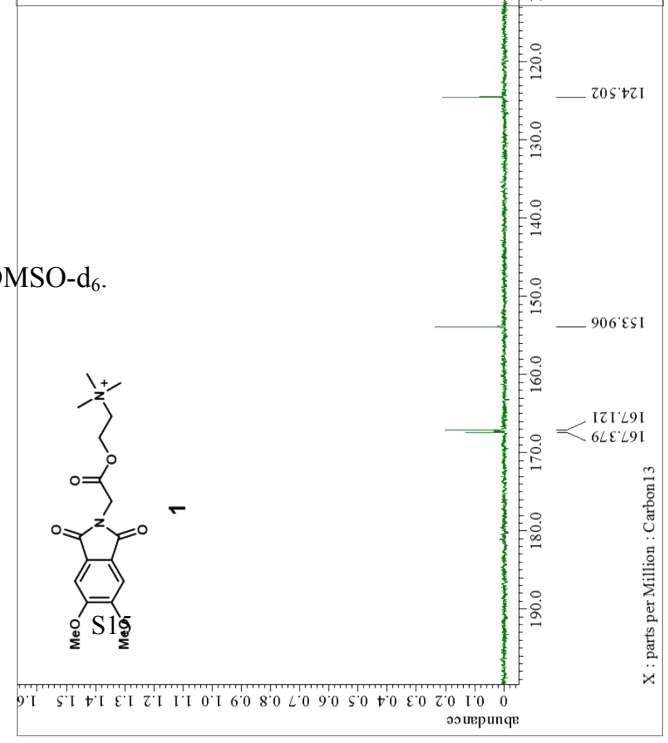


Figure S18. ^{13}C-NMR spectrum of **1** in DMSO-d_6.

Figure S19. ESI-Mass spectrum of **1**.

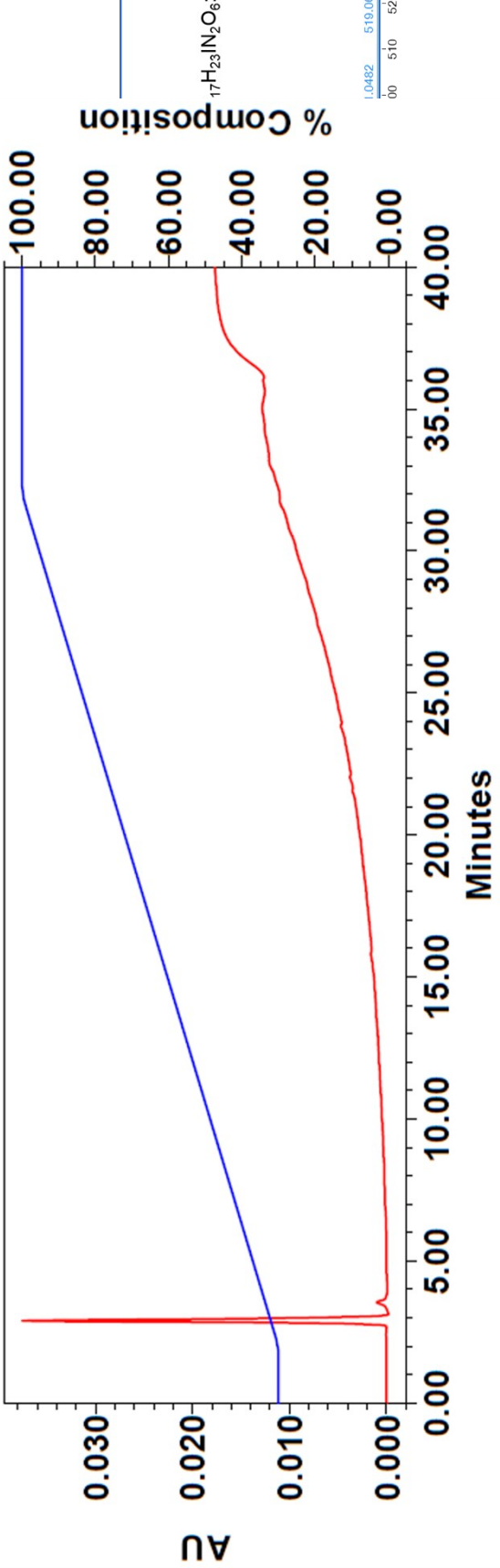


Figure S20. HPLC chromatogram of **1**.

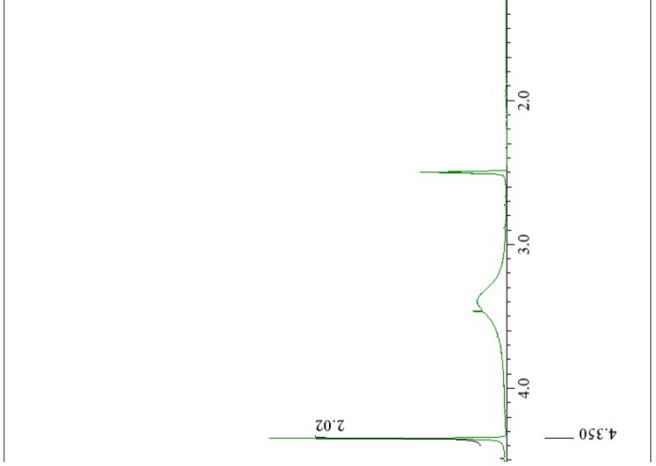


Figure S21. ^{1}H-NMR spectrum of **S2** in D

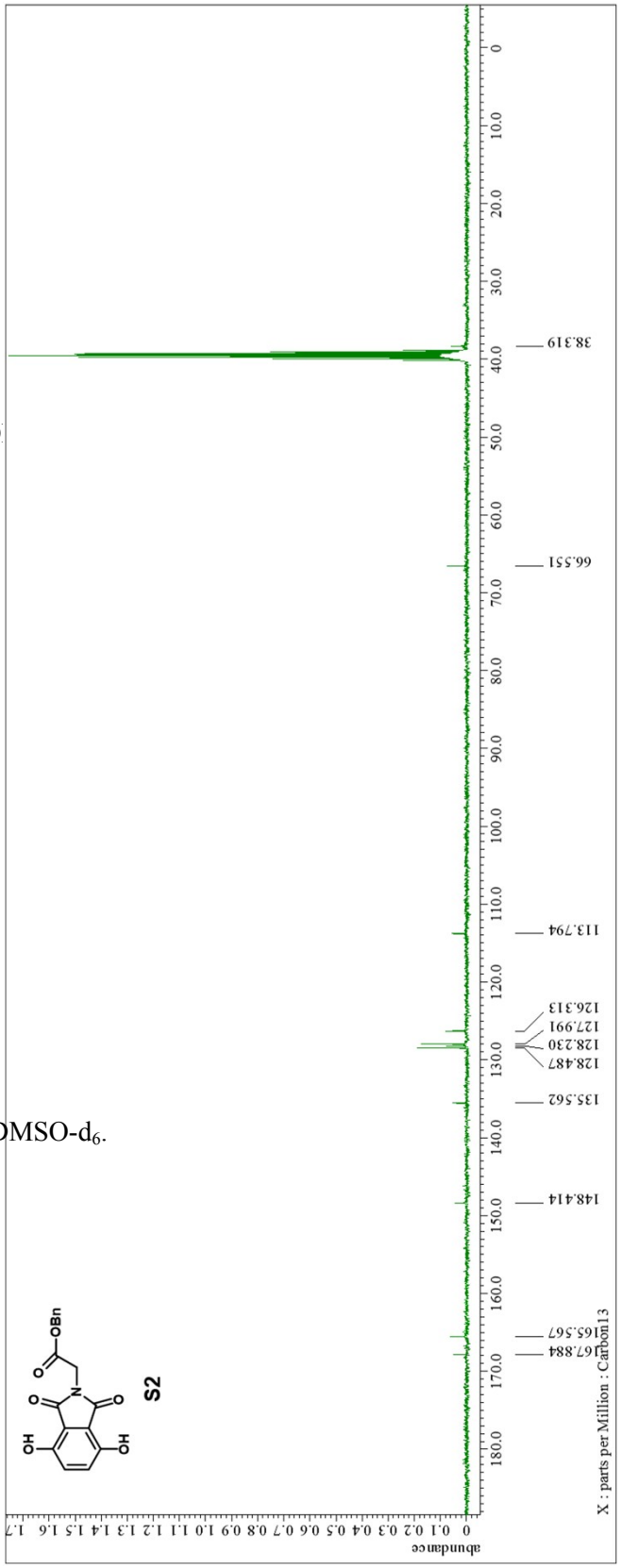


Figure S22. ^{13}C-NMR spectrum of **S2** in DMSO-d_6.

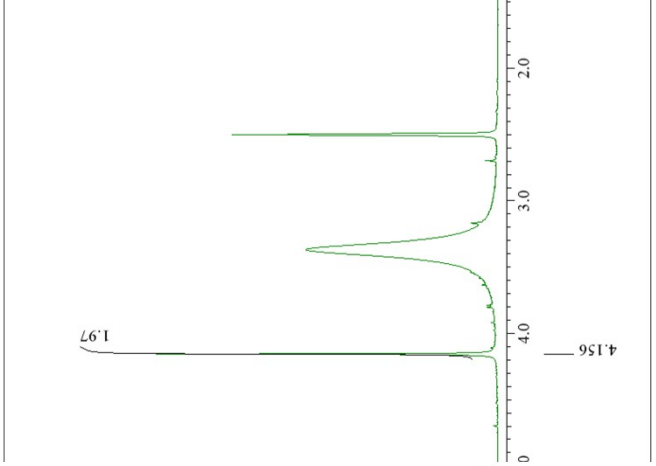


Figure S23. ^{1}H-NMR spectrum of **S1** in D

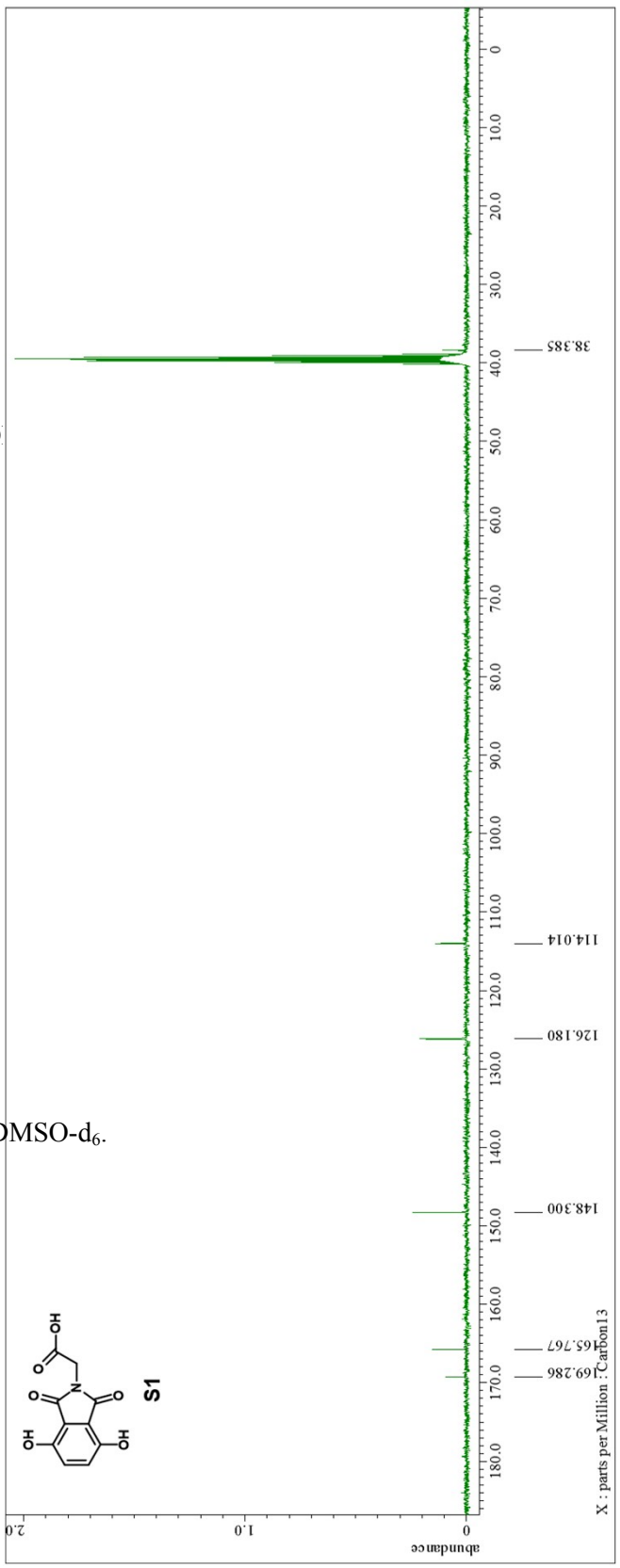


Figure S24. ^{13}C-NMR spectrum of **S1** in DMSO-d_6.