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Tailoring the Positive and Negative Solvatochromism for Chalcone Analogues to Detect Heterozygous Protein Co-aggregation

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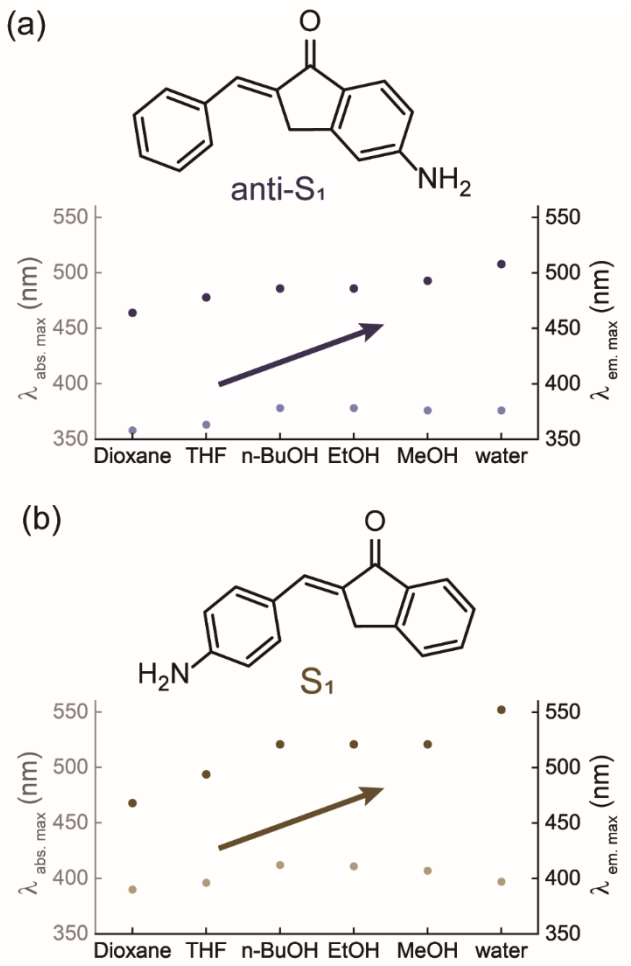


Fig. S1 Solvatochromism of anti-S_1 and S_1 in fluorescence emission wavelength. Both anti-S_1 and S_1 showed a red-shift in their fluorescence emission wavelength as polarity increased. (a) Anti-S_1 exhibited slight red shift of its absorption and fluorescence emission wavelength with increasing polarity. (b) Compared to anti-S_1, S_1 exhibited slightly intensive red shift in absorption and fluorescence emission wavelength with increasing polarity.

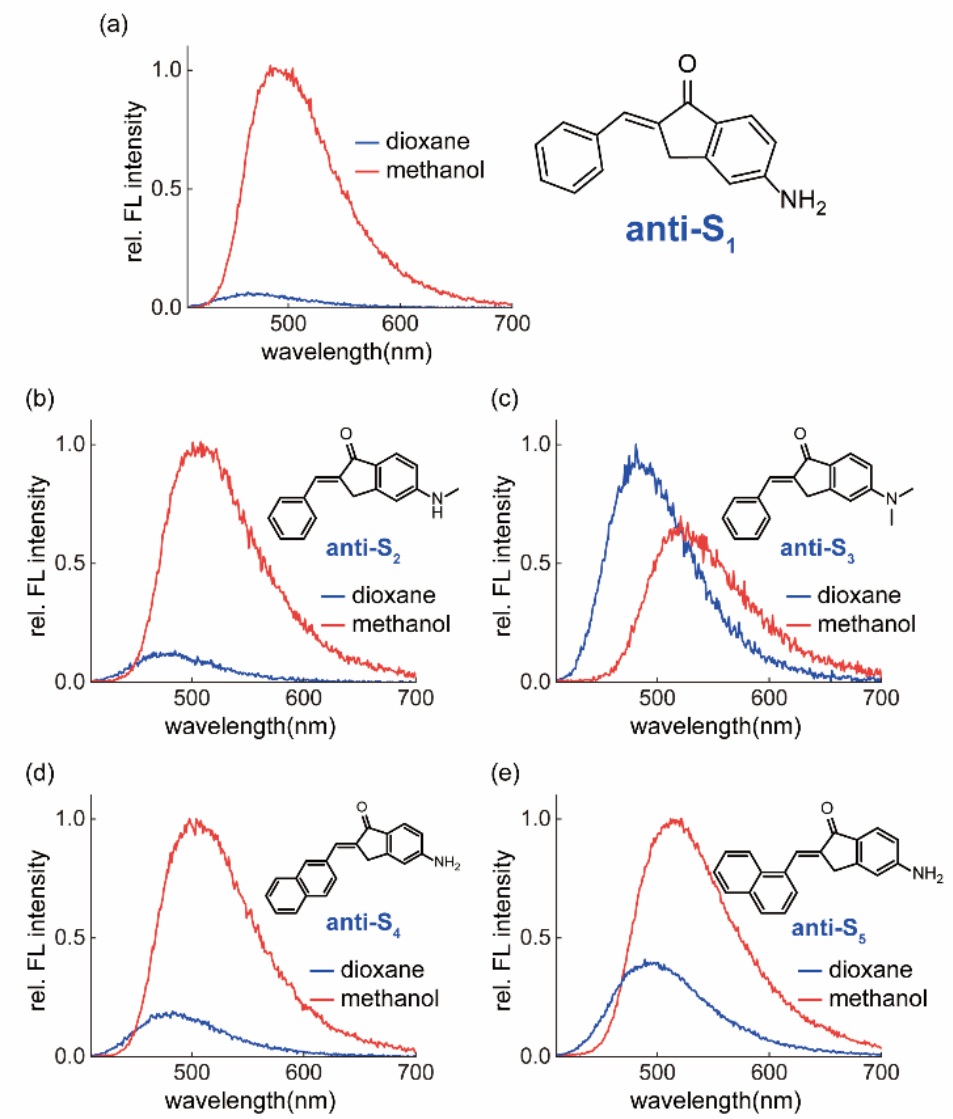


Fig. S2 The solvatochromic properties of anti-S group probes. anti-S series (10 μM) were prepared in non-polar dioxane and polar methanol. Fluorescence emission spectra were collected by using 358 nm for anti-S_1, 368 nm for anti-S_2, 381 nm for anti-S_3, 366 nm for anti-S_4, 365 nm for anti-S_5, as excitation wavelength. The fluorescence spectra were measured by Tecan Spark Fluorescence Plate Reader using BeyoGold™ 96-Well Black Opaque plates.

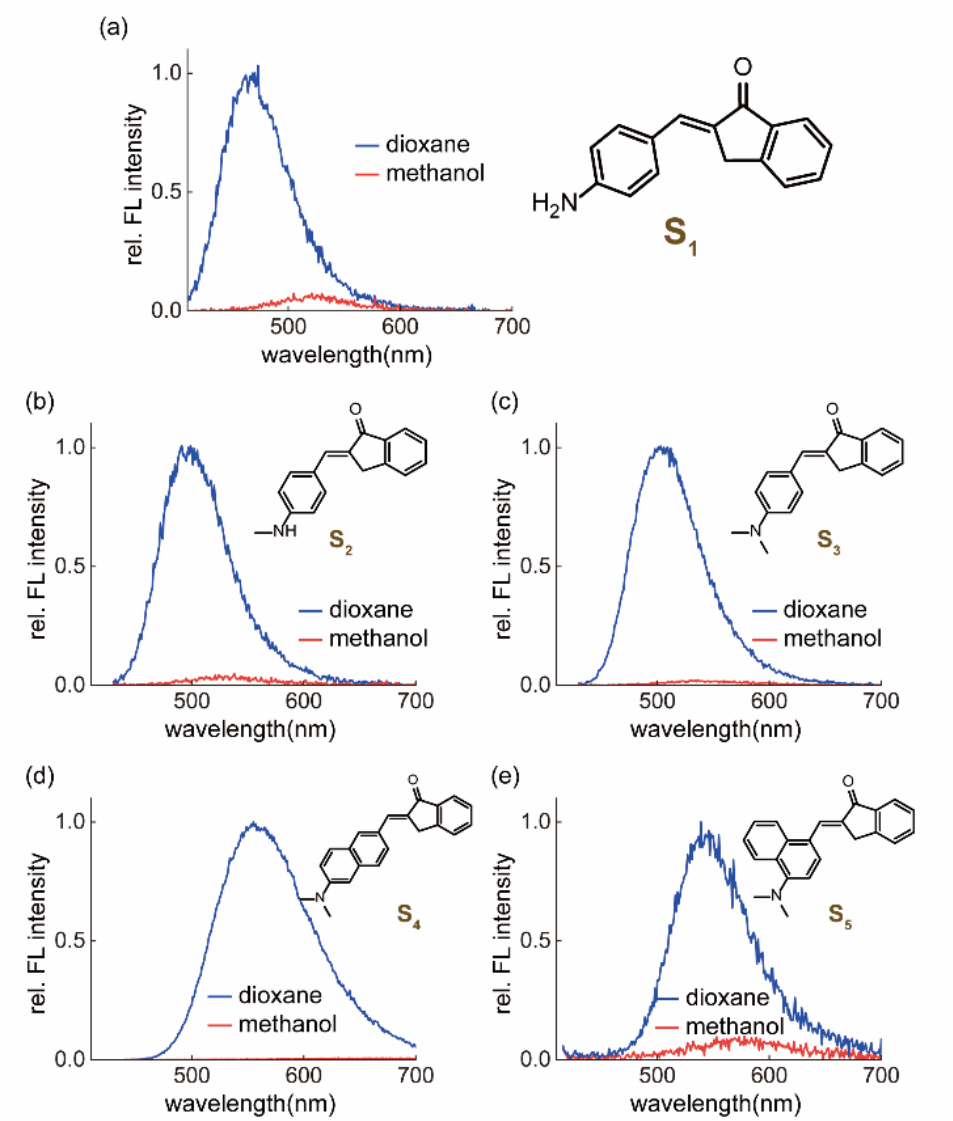


Fig. S3 The solvatochromic properties of S group probes. S series (10 μM) were dissolved in non-polar dioxane and polar methanol. Fluorescence emission spectra were collected by using 390 nm for S_1, 410 nm for S_2, 415 nm for S_3, 423 nm for S_4, 401 nm for S_5 as excitation wavelength. The fluorescence spectra were measured by Tecan Spark Fluorescence Plate Reader using BeyoGold™ 96-Well Black Opaque plates.

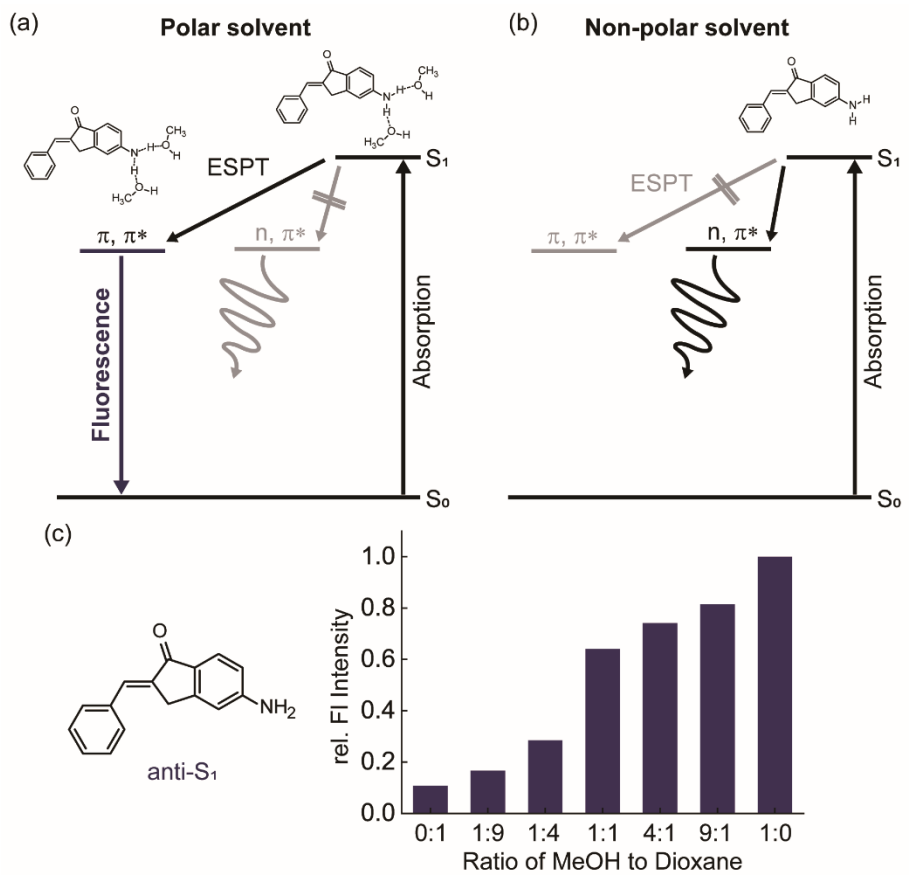


Fig. S4 Schematic of the Excited-State Proton Transfer (ESPT) process in (a) polar solvent and (b) non-polar solvent. (a) anti-S_1 may undergo ESPT process in a polar solvent and reach an emissive excited state, which induces fluorescence emission. (b) ESPT process is inhibited in a polar solvent and anti-S_1 reached a non-emissive excited state, which quenches its fluorescence. (c) The fluorescence intensity of anti-S_1 increases as the solvent becomes more polar.

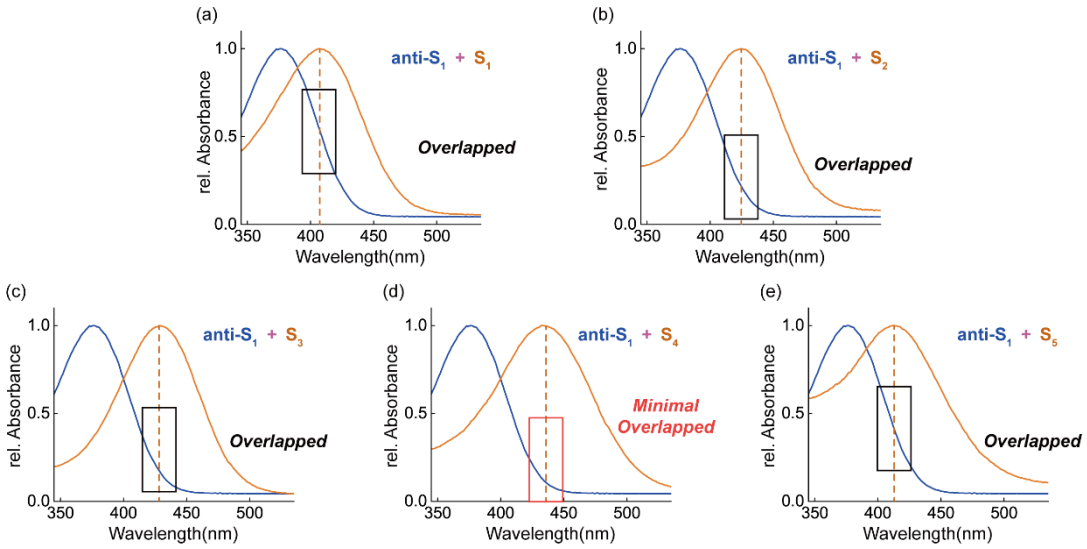


Fig. S5 anti-S_1 has minimal overlap with the maximum absorption of S_4. anti-S_1 could hardly be excited under the maximum absorption of S_4, compared to other four S series molecules. Therefore, we can use 360 nm for excitation of anti-S_1 and 468 nm for excitation of S_4 at the same time in mixed system. Under these conditions, we can avoid both fluorophores being excited when excited at 360 nm or 468 nm to a greatest extent. we utilized 360 nm wavelength to collect the emission intensity of anti-S_1, at which S_4 was not significantly excited. Therefore, S_4 and anti-S_1 pair was selected to monitor dual protein co-aggregation process.

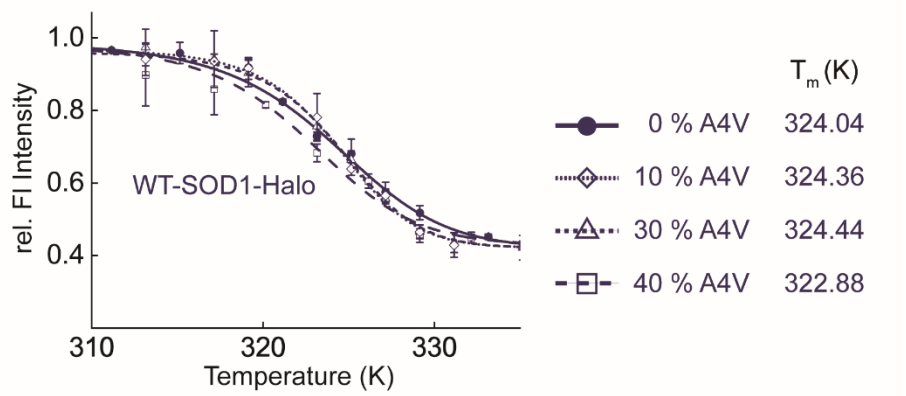


Fig. S6 The stoichiometry of co-aggregation experiment can affect their impact on protein stability. The destabilizing effect of A4V-SOD1-Halo on WT-SOD1-Halo proteins exhibits when it reaches 40 % in stoichiometry. The experiment details are showed below. 40 μL solution with purified WT–SOD1-Halo (90-150 μM) and anti-S_1-Halo (6 μM) was incubated together at room temperature for 10 minutes, 40 μL solution with purified A4V-SOD1-Halo (0-60 μM) and S_4-Halo (6 μM) was incubated together at room temperature for 10 minutes simultaneously. Above-mentioned two protein samples were mixed with 40 μL EDTA (0.15 M, to accelerate aggregation), and then incubated for 5 minutes at different temperatures (35 °C to 66 °C). The incubated protein mixtures (100 μL) were pipetted into 96-Well Black Opaque plates and collected the fluorescence emission from 500 nm to 800 nm by using 468 nm as the excitation wavelength. In addition, fluorescence emission from 400 nm to 800 nm was also collected by using 360 nm as the excitation wavelength. For each temperature, the experiments were repeated three times.

Table S1 The photophysical parameters of S and anti-S series in non-polar dioxane.

Compound	λ_{abs} (nm)	λ_{em} (nm)	Φ_f (%)	ε ($M^{-1}cm^{-1}$) [a]
S_1	390	468	1.01	26960
S_2	410	497	2.26	16930
S_3	415	506	3.39	36280
S_4	423	555	37.20	18958
S_5	432	540	1.13	12530
anti-S_1	358	464	0.09	32370
anti-S_2	368	483	0.66	6723
anti-S_3	381	481	1.01	25994
anti-S_4	366	485	0.12	33829
anti-S_5	401	539	0.21	5117

[a] Molar extinction coefficient was measured at the maximal absorption wavelength of each molecule.

Table S2 The photophysical parameters of S and anti-S series in polar methanol.

Compound	λ_{abs} (nm)	λ_{em} (nm)	Φ_f (%)	ε ($M^{-1}cm^{-1}$) [a]
S_1	407	521	0.28	26450
S_2	408	532	0.45	14666
S_3	418	537	0.21	32891
S_4	424	670	0.31	18365
S_5	433	576	0.10	13573
anti-S_1	376	493	14.76	31850
anti-S_2	387	510	19.54	7462
anti-S_3	398	520	2.85	25835
anti-S_4	386	504	10.01	31597
anti-S_5	413	573	4.7	11650

[a] Molar extinction coefficient was measured at the maximal absorption wavelength of each molecule.

Experimental Method Section：

1. Protein purifications.

SOD1-Halo-His plasmid was transformed into BL21 DE3 *E. coli* cells. Cells were grown to OD_{600} at 0.6-0.8 before induced by IPTG (0.5 mM) at 18 °C overnight. Cultured cells were harvested and resuspended in resuspension buffer (50 mM Tris,100 mM NaCl, pH = 8.00). Cells expressing recombinant proteins were thawed and lysed by sonication at 4 °C. Lysed cells were centrifuged for 60 min at 13,000 rpm. The supernatant was collected and loaded into a 10 mL Ni-NTA column and eluted with buffer A (50 mM Tris, 100 mM NaCl, pH = 8.00) to remove non-bonded impurities. The desired proteins were then eluted by gradient addition of buffer B (50 mM Tris, 100 mM NaCl, 500 mM imidazole, pH = 8.00). The protein fractions were identified by SDS-PAGE analysis, then combined and concentrated. The Ni-NTA column treated proteins were further purified through dialysis against buffer A (50 mM Tris, 100 mM NaCl, pH = 8.00). The protein containing fractions were identified by SDS-PAGE gel analysis, pooled, and concentrated. No significant impurity was identified and purity of proteins were estimated to be 98% based on SDS-PAGE electrophoresis analysis.

2. Heat induced SOD1-Halo aggregation and fluorescence measurement.

SOD1-Halo protein (75 μM) and anti-S_1-Halo or S_4-Halo (3 μM) were mixed together in buffer A (50 mM Tris, 100 mM NaCl, pH = 8.00) for 10 minutes. EDTA was then add to the mixture to destruct protein structure. The solution mixture was incubated at 66 °C for 5 min. The fluorescent spectra of aggregated protein samples were collected by Tecan Spark Fluorescence Plate Reader using 96-Well Black Opaque plates (for anti-S_1-Halo: ex. 360 nm, em. 530 nm; for S_4-Halo: ex. 468 nm, em. 570 nm).

To measure the linear range and detection limits, purified WT-SOD1-Halo protein and anti-S_1-Halo or S_4-Halo (1 mM) were prepared. Mixing WT-SOD1-Halo with varying concentration of molecules (final concentration was 0.2-10 μM for S_4-Halo and 0.5-15 μM for anti-S_1-Halo,) in buffer A (50 mM Tris, 100 mM NaCl, pH = 8.00) for 10 min then added EDTA, the finely prepared samples were incubated at 66 °C for 5 min to induce SOD1 aggregation. For S_4-Halo, fluorescence intensity was measured by exciting aggregated samples at 468 nm and fluorescence emission was collected from 500 nm to 800 nm. For anti-S_1-Halo, fluorescence intensity was measured by exciting the samples at 360 nm and fluorescence emission was collected from 400 nm to 800 nm.

3. SOD1-Halo thermal shift assay.

80 μL solution with purified WT-, A4V-, V31A-, G85R- or G93A-SOD1-Halo (75 μM) and S_4-Halo (6 μM) were mixed at room temperature for 10 minutes, and then 40 μL EDTA (0.1 M) was introduced to incubate for 5 minutes at different temperatures (25 °C to 66 °C). The incubated mixtures (100 μL) were pipetted into 96-Well Black Opaque plates and collected the fluorescence emission from 500 nm to 800 nm by using 468 nm as the excitation wavelength. For each temperature, the experiments were repeated three times.

80 μL solution with purified WT-, A4V-, V31A-, G85R- or G93A-SOD1-Halo (75 μM) and anti-S_1-Halo (6 μM) were mixed at room temperature for 10 minutes, and then 40 μL EDTA (0.1 M) was added to incubate for 5 minutes at different temperatures (25 °C to 66 °C). The incubated mixtures (100 μL) were pipetted into 96-Well Black Opaque plates and collected the fluorescence emission from 400 nm to 800 nm by using 360 nm as the excitation wavelength. For each temperature, the experiments were repeated three times.

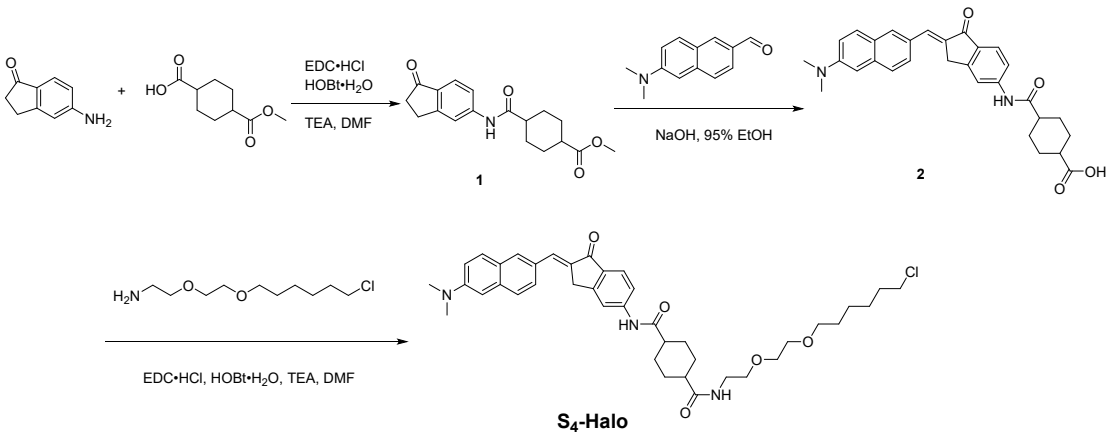
4. Dual-color thermal shift assay for WT- and Mut-SOD1-Halo co-aggregation.

40 μL solution with purified WT–SOD1-Halo (75 μM) and anti-S_1-Halo (6 μM) was incubated together at room temperature for 10 minutes, 40 μL solution with purified A4V- or V31A-SOD1-Halo (75 μM) and S_4-Halo (6 μM) was incubated together at room temperature for 10 minutes simultaneously. Above-mentioned two protein samples were mixed together with 40 μL EDTA (0.1 M), and then incubated for 5 minutes at

different temperatures (25 °C to 66 °C). The incubated protein mixtures (100 μL) were pipetted into 96-Well Black Opaque plates and collected the fluorescence emission from 500 nm to 800 nm by using 468 nm as the excitation wavelength. In addition, fluorescence emission from 400 nm to 800 nm was also collected by using 360 nm as the excitation wavelength. For each temperature, the experiments were repeated three times.

Synthetic Methods and Schemes:

Scheme S1. Methods of connecting S_4 with rigid and Halo-tag linker. [1]

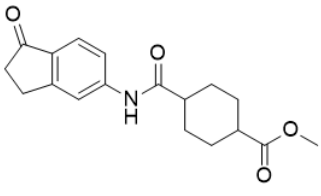


2,3-dihydro-1H-inden-1-one (1.0 equiv., 1.0 mmol, 294.4 mg) was dissolved in 20 mL anhydrous DMF, and then 4-(Methoxycarbonyl)cyclohexanecarboxylic acid (1.0 equiv., 2.0 mmol, 372.4 mg), 1-Hydroxybenzotriazole hydrate (HOBt·H_2O) (3.0 equiv., 6.0 mmol, 920.0 mg), triethylamine (3.0 equiv., 6.0 mmol, 840.0 μL) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) (3.0 equiv., 6.0 mmol, 1060.0 mg) were introduced to the reaction mixture in one portion. The reaction mixture was stirred for 12 hours in dark followed by extraction with water (200 mL) and DCM (3 × 200 mL). The combined organic phase was concentrated *in vacuo* and further purified *via* flash chromatography by using 5% MeOH in DCM as eluent to obtain the compound **1.**

6-(Dimethylamino)-2-naphthaldehyde (1.0 equiv., 1.0 mmol, 186.2 mg), compound **1** (1.0 equiv., 1 mmol) and catalytic amount of 95 % NaOH (0. 1 equiv.) were added to ethanol (5.0 mL), and the mixture was then stirred overnight at 52 °C in argon atmosphere. The reaction mixture was then cooled down to room temperature, and the formed solid was isolated *via* suction filtration. The solid was rinsed with cold ethanol (20.0 ml) and yield desired product compound **2**.

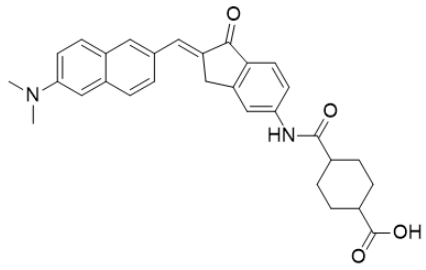
Compound **2** (1.0 equiv., 0.1 mmol) was dissolved in 1.0 mL anhydrous DMF, and then Halo-Tag linker (1.0 equiv., 0.1 mmol, 26.0 mg), 1-Hydroxybenzotriazole hydrate (HOBt·H_2O) (3.0 equiv., 0.3 mmol, 46.0 mg), triethylamine (3.0 equiv., 0.3 mmol, 42.0 μL) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) (3.0 equiv., 0.3 mmol, 58.0 mg) were introduced to the reaction mixture in one portion. The reaction mixture was stirred for 12 hours in dark followed by extraction with water (200 mL) and DCM (3 × 200 mL). The combined organic phase was concentrated *in vacuo* and further purified *via* flash chromatography by using 5% MeOH in DCM as eluent to obtain the compound **S_4-Halo** probes.

Compound **1**



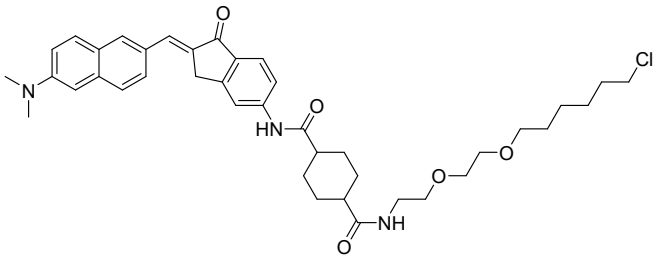
Pale brown foams (47.1 %). ^{1}H-NMR (600 MHz, DMSO-d^6) δ 10.24 (s, 1H), 7.93 (s, 1H), 7.56 (d, J = 8.4 Hz, 1H), 7.51 (dd, J = 8.4, 1.8 Hz, 1H), 3.60 (s, 3H), 3.06 – 3.01 (m, 2H), 2.59 – 2.54 (m, 2H), 2.39 – 2.29 (m, 2H), 1.98 (dd, J = 13.2, 3.7 Hz, 2H), 1.89 (dd, J = 13.5, 3.6 Hz, 2H), 1.47 (qd, J = 13.0, 3.2 Hz, 2H), 1.37 (qd, J = 13.0, 3.4 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d^6) δ 205.09, 175.67, 175.01, 157.26, 145.64, 131.95, 124.29, 118.71, 116.03, 51.82, 44.62, 41.99, 36.41, 28.43, 28.23, 25.91, 18.53, 17.18. HRMS (ESI$^+$) calcd. for $C_{18}H_{21}NO_4$ $[M+H]^+$: 316.1549, found $[M+H]^+$: 316.1543.

Compound **2**



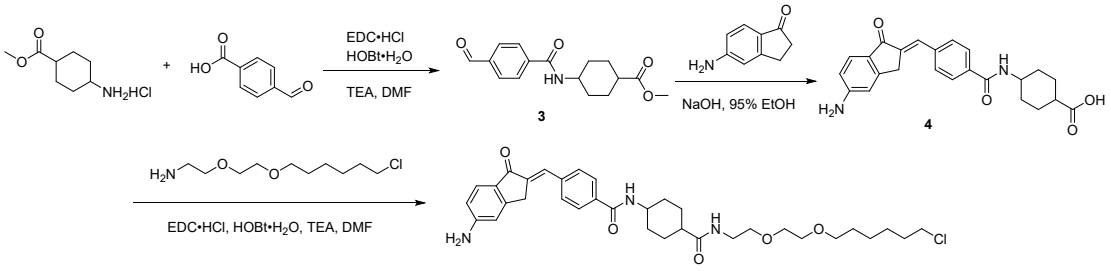
Red solid (55.6 %). ^{1}H-NMR (600 MHz, DMSO-d^6) δ 12.10 (s, 1H), 10.34 (s, 1H), 8.14 (s, 1H), 8.09 (s, 1H), 7.86 (d, *J* = 9.1 Hz, 1H), 7.73 (d, *J* = 13.0 Hz, 3H), 7.62 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.56 (d, *J* = 2.2 Hz, 1H), 7.28 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.97 (d, *J* = 2.5 Hz, 1H), 4.16 (s, 2H), 3.07 (s, 6H), 2.39 (td, *J* = 13.6, 11.9, 3.5 Hz, 1H), 2.23 (ddt, *J* = 12.4, 8.8, 3.8 Hz, 1H), 2.02 – 1.98 (m, 2H), 1.92 (d, *J* = 14.6 Hz, 2H), 1.50 (qd, *J* = 13.2, 3.5 Hz, 2H), 1.37 (qd, *J* = 12.9, 3.4 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d^6) δ 190.43, 179.59, 175.68, 175.23, 165.42, 165.30, 156.41, 153.39, 138.86, 138.82, 138.45, 138.36, 135.13, 134.98, 130.33, 128.73, 128.25, 126.37, 126.16, 114.69, 108.48, 60.21, 48.35, 42.16, 32.57, 32.16, 31.59, 29.58, 28.20, 14.58. HRMS (ESI$^+$) calcd. for $C_{30}H_{30}N_2O_4$ $[M+H]^+$: 483.2284, found $[M+H]^+$: 483.2294.

Compound **S_4-Halo**



Orange foams (36.5 %). ^{1}H-NMR (600 MHz, DMSO-d^6) δ 10.32 (s, 1H), 8.14 (s, 1H), 8.09 – 8.07 (m, 1H), 7.86 (d, *J* = 9.1 Hz, 1H), 7.82 (t, *J* = 5.7 Hz, 1H), 7.76 – 7.71 (m, 3H), 7.62 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.57 – 7.55 (m, 1H), 7.28 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.97 (d, *J* = 2.5 Hz, 1H), 4.18 – 4.14 (m, 2H), 3.63 (t, *J* = 6.6 Hz, 2H), 3.51 (dd, *J* = 6.3, 3.8 Hz, 2H), 3.48 (dd, *J* = 5.7, 3.2 Hz, 2H), 3.39 (dt, *J* = 16.4, 6.3 Hz, 4H), 3.19 (q, *J* = 5.8 Hz, 2H), 2.39 (ddd, *J* = 15.1, 9.4, 3.6 Hz, 1H), 2.15 (ddt, *J* = 11.2, 6.8, 3.5 Hz, 1H), 1.94 – 1.88 (m, 2H), 1.83 – 1.78 (m, 2H), 1.74 – 1.68 (m, 2H), 1.50 (dd, *J* = 8.2, 6.4 Hz, 2H), 1.46 – 1.42 (m, 2H), 1.41 – 1.37 (m, 2H), 1.34 – 1.30 (m, 2H), 1.24 (d, *J* = 4.3 Hz, 2H). ^{13}C-NMR (151 MHz, DMSO-d^6) δ 192.3, 175.4, 175.2, 151.9, 149.9, 145.6, 135.7, 133.8, 133.2, 133.0, 130.2, 128.6, 128.0, 126.9, 126.2, 119.0, 115.7, 105.5, 70.7, 70.1, 69.9, 69.6, 45.8, 44.8, 43.6, 40.5, 38.9, 32.7, 32.5, 29.6, 28.8, 28.7, 26.6, 25.4. HRMS (ESI$^+$) calcd. for $C_{40}H_{51}N_3O_5Cl$ $[M+H]^+$: 688.3517, found $[M+H]^+$: 688.3524.

Scheme S2. Methods of connecting anti-S_1 with rigid and Halo-tag linker.



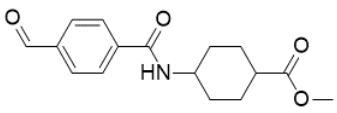
anti-S_1-Halo

Methyl 4-aminocyclohexanecarboxylate hydrochloride (1.0 equiv., 2.0 mmol, 387.4 mg) was dissolved in 20 mL anhydrous DMF, and then 4-formylbenzoic acid (1.0 equiv., 2.0 mmol, 300.6 mg), 1-Hydroxybenzotriazole hydrate (HOBt·H_2O) (3.0 equiv., 6.0 mmol, 920.0 mg), triethylamine (3.0 equiv., 6.0 mmol, 840.0 μL) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) (3.0 equiv., 6.0 mmol, 1060.0 mg) were introduced to the reaction mixture in one portion. The reaction mixture was stirred for 12 hours in dark followed by extraction with water (200 mL) and DCM (3 × 200 mL). The combined organic phase was concentrated *in vacuo* and further purified *via* flash chromatography by using 5% MeOH in DCM as eluent to obtain the compound **3.**

2,3-dihydro-1H-inden-1-one (1.0 equiv., 1.0 mmol, 147.2 mg), compound **1** (1.0 equiv., 1 mmol) and catalytic amount of 95 % NaOH (0. 1 equiv.) were added to ethanol (5.0 mL), and the mixture was then stirred overnight at 52 °C in argon atmosphere. The reaction mixture was then cooled down to room temperature, and the formed solid was isolated *via* suction filtration. The solid was rinsed with cold ethanol (20.0 ml) and yield desired product compound **4**.

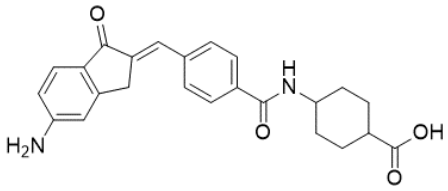
Compound **4** (1.0 equiv., 0.1 mmol) was dissolved in 1.0 mL anhydrous DMF, and then Halo-Tag linker (1.0 equiv., 0.1 mmol, 26.0 mg), 1-Hydroxybenzotriazole hydrate (HOBt·H_2O) (3.0 equiv., 0.3 mmol, 46.0 mg), triethylamine (3.0 equiv., 0.3 mmol, 42.0 μL) and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) (3.0 equiv., 0.3 mmol, 58.0 mg) were introduced to the reaction mixture in one portion. The reaction mixture was stirred for 12 hours in dark followed by extraction with water (200 mL) and DCM (3 × 200 mL). The combined organic phase was concentrated *in vacuo* and further purified *via* flash chromatography by using 5% MeOH in DCM as eluent to obtain the compound **anti-S_1-Halo** probes.

Compound **3**



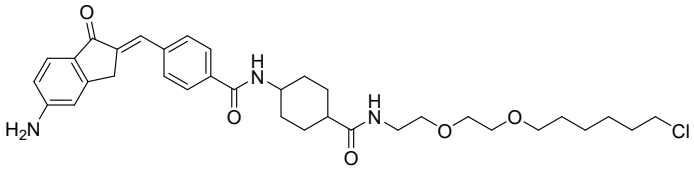
Brown foams (57.0 %). ^{1}H NMR (600 MHz, DMSO-d^6) δ 10.09 (s, 1H), 8.48 (d, J = 7.8 Hz, 1H), 8.05 – 7.98 (m, 4H), 3.78 (dtd, J = 11.3, 7.5, 3.8 Hz, 1H), 3.61 (s, 3H), 2.34 – 2.25 (m, 1H), 2.01 – 1.89 (m, 4H), 1.49 – 1.35 (m, 4H). ^{13}C NMR (151 MHz, DMSO-d^6) δ 193.35, 175.60, 165.14, 140.25, 138.12, 129.77, 128.48, 51.80, 48.46, 42.04, 31.54, 28.12. HRMS (ESI$^+$) calcd for $C_{16}H_{19}NO_4$ $[M+H]^+$: 290.1392, found $[M+H]^+$: 290.1392.

Compound **4**



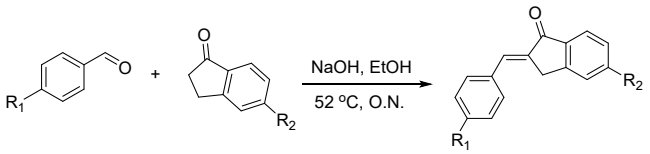
Yellow powder (74.4 %). ^{1}H-NMR (600 MHz, DMSO-d^6) δ 8.23 (d, J = 8.0 Hz, 1H), 7.91 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 8.1 Hz, 2H), 7.47 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 2.8 Hz, 1H), 6.64 (s, 1H), 6.61 (d, J = 8.4 Hz, 1H), 6.45 (s, 2H), 3.93 (s, 2H), 3.69 (qt, J = 7.7, 4.3 Hz, 1H), 1.88 – 1.78 (m, 4H), 1.73 – 1.66 (m, 1H), 1.29 (q, J = 10.6, 10.1 Hz, 5H). ^{13}C NMR (151 MHz, methanol-d^4) δ 193.93, 183.55, 176.57, 151.95, 149.78, 145.11, 135.85, 134.48, 132.68, 131.92, 129.48, 128.44, 126.37, 124.33, 118.82, 115.74, 105.10, 46.49, 45.60, 39.20, 32.21, 29.79, 29.14, 28.91. HRMS (ESI$^+$) calcd. for $C_{24}H_{24}N_2O_4$ $[M+H]^+$: 405.1814, found $[M+H]^+$: 405.1808.

Compound **anti-S_1-Halo**



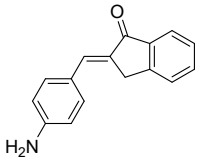
Yellow foams (42.8 %). ^{1}H-NMR (600 MHz, DMSO-d^6) δ 8.30 (d, *J* = 7.9 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.81 – 7.75 (m, 3H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.34 (d, *J* = 2.5 Hz, 1H), 6.64 (s, 1H), 6.61 (d, *J* = 8.5 Hz, 1H), 6.45 (s, 2H), 3.93 (d, *J* = 2.2 Hz, 2H), 3.75 (ddt, *J* = 15.6, 11.5, 5.6 Hz, 1H), 3.63 (t, *J* = 6.6 Hz, 2H), 3.51 (dd, *J* = 6.0, 3.6 Hz, 2H), 3.48 (d, *J* = 5.0 Hz, 2H), 3.39 (dt, *J* = 15.6, 6.3 Hz, 4H), 3.19 (q, *J* = 5.7 Hz, 2H), 2.11 (tt, *J* = 11.8, 3.5 Hz, 1H), 1.92 – 1.87 (m, 2H), 1.80 – 1.75 (m, 2H), 1.71 (m, 2H), 1.50 (dd, *J* = 14.3, 7.1 Hz, 2H), 1.48 – 1.43 (m, 2H), 1.41 – 1.34 (m, 4H), 1.33 – 1.29 (m, 2H). ^{13}C-NMR (151 MHz, DMSO-d^6) δ 190.4, 175.4, 165.3, 156.4, 153.4, 138.9, 138.5, 135.1, 130.4, 128.2, 126.4, 126.2, 114.7, 108.5, 70.7, 70.1, 69.9, 69.6, 48.5, 45.8, 43.6, 38.9, 32.5, 32.15, 32.0, 29.6, 28.8, 26.6, 25.4. HRMS (ESI$^+$) calcd. for $C_{34}H_{45}N_3O_5Cl$ $[M+H]^+$: 610.3047, found $[M+H]^+$: 610.3054.

Scheme S3. General methods of the synthesis of S series and anti-S series. [2]



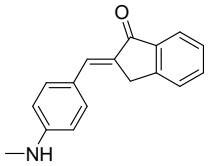
Para-substituted benzaldehyde (1.0 mmol, 1.0 eq.), 2,3-dihydro-1H-inden-1-one or its analogues (1 mmol, 1.0 eq.) and catalytic amount of NaOH (0.1 eq.) were added to ethanol (5.0 mL), and the mixture was then stirred overnight at 52 °C in argon atmosphere. The reaction mixture was then cooled down to room temperature, and the formed solid was isolated *via* suction filtration. The solid was rinsed with cold ethanol (20.0 ml) and yield desired product.

Compound **S_1**



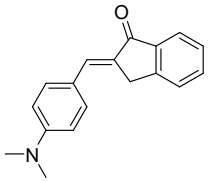
Yellow powder (80.0%). ^{1}H-NMR (700 MHz, DMSO-d^6) δ 7.74 (d, *J* = 7.5 Hz, 1H), 7.69 – 7.64 (m, 2H), 7.52 – 7.49 (m, 2H), 7.46 (ddd, *J* = 8.0, 6.2, 1.9 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 6.74 – 6.63 (m, 2H), 5.94 (s, 2H), 4.01 (d, *J* = 2.0 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d^6) δ 192.36, 150.71, 148.89, 137.48, 133.94, 133.41, 132.53, 128.10, 126.84, 125.88, 122.57, 121.58, 113.26. HRMS (ESI$^+$) calcd for $C_{16}H_{14}NO$ [M+H]$^+$: 236.1075, found [M+H]$^+$: 236.1067.

Compound **S_2**



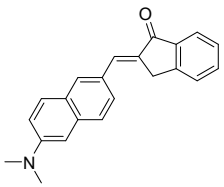
Yellow powder (79.0%). ^{1}H-NMR (700 MHz, Chloroform-d) δ 7.83 (d, *J* = 7.6 Hz, 1H), 7.56 (s, 1H), 7.49 (m, 4H), 7.34 (t, *J* = 7.4 Hz, 1H), 6.58 (d, *J* = 8.2 Hz, 2H), 3.92 (s, 2H), 2.83 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d^6) δ 193.35, 152.08, 149.93, 138.56, 134.97, 134.47, 133.49, 130.11, 129.20, 129.14, 127.91, 126.95, 123.63, 122.53, 112.21, 32.66, 29.71. HRMS (ESI$^+$) calcd for $C_{17}H_{15}NO$ [M+H]$^+$: 250.1226, found [M+H]$^+$: 250.1223.

Compound **S_3**



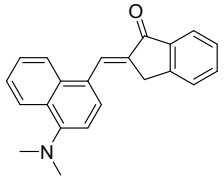
Yellow powder (83.0%). The spectra were consistent with those reported previously.[2] ^{1}H-NMR (700 MHz, DMSO-d^6) δ 7.75 (d, *J* = 7.5 Hz, 1H), 7.67 (d, *J* = 5.4 Hz, 2H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 5.2 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 4.03 (s, 2H), 3.02 (s, 6H). HRMS (ESI$^+$) calcd for $C_{18}H_{18}NO$ [M+H]$^+$: 264.1388, found [M+H]$^+$: 264.1375.

Compound **S_4**



Orange powder (55.0%). ^{1}H-NMR (700 MHz, DMSO-d^6) δ 8.18 (s, 1H), 7.87 (d, *J* = 9.0 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 4.8 Hz, 2H), 7.73 (d, *J* = 4.0 Hz, 2H), 7.65 (s, 1H), 7.50 (dt, *J* = 8.4, 3.9 Hz, 1H), 7.29 (dd, *J* = 9.2, 2.3 Hz, 1H), 6.98 (s, 1H), 4.23 (s, 2H), 3.08 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d^6) δ 192.59, 149.32, 148.94, 137.02, 134.76, 133.97, 133.27, 132.12, 131.24, 129.15, 127.42, 127.02, 126.91, 126.03, 125.87, 125.11, 122.85, 115.90, 104.41, 39.43, 31.57. HRMS (ESI$^+$) calcd for $C_{22}H_{19}NO$ [M+H]$^+$: 314.1539, found [M+H]$^+$: 314.1572.

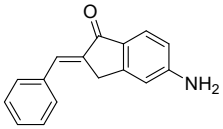
Compound **S_5**



Yellow powder (62.0%). ^{1}H-NMR (700 MHz, DMSO-d^6) δ 8.26 (s, 1H), 8.21 (dd, *J* = 11.6, 8.5 Hz, 2H), 7.97 (d, *J* = 7.9 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.71 (t, *J* = 7.4 Hz, 1H), 7.67 (d, *J* = 7.6 Hz, 1H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.49 (t, *J* = 7.4 Hz, 1H), 7.19 (d, *J* = 7.9 Hz, 1H), 4.11 (s, 2H), 2.92 (s, 6H). ^{13}C NMR (151 MHz,

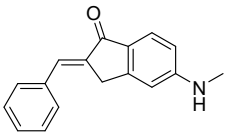
DMSO-d^6) δ 193.44, 153.01, 150.66, 138.09, 135.58, 135.17, 133.83, 129.56, 128.96, 128.15, 128.14, 127.45, 127.12, 125.81, 125.41, 125.29, 124.18, 124.08, 113.77, 45.01, 32.38. HRMS (ESI$^+$) calcd for $C_{22}H_{19}NO$ $[M+H]^+$: 314.1539, found $[M+H]^+$: 314.1603.

Compound **anti-S_1**



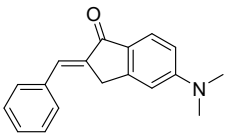
Pale yellow foams (85.0%). The spectra were consistent with those reported previously.[2] ^{1}H-NMR (700 MHz, DMSO-d^6) δ 7.76 – 7.69 (m, 2H), 7.48 (t, *J* = 7.9 Hz, 3H), 7.43 – 7.39 (m, 1H), 7.32 (d, *J* = 2.2 Hz, 1H), 6.64 (d, *J* = 1.9 Hz, 1H), 6.61 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.41 (s, 2H), 3.91 (d, *J* = 2.2 Hz, 2H). HRMS (ESI$^+$) calcd for $C_{16}H_{14}NO$ $[M+H]^+$: 236.1075, found $[M+H]^+$: 236.1066.

Compound **anti-S_2**



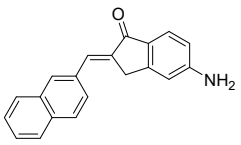
Yellow powder (52.0%). ^{1}H-NMR (700 MHz, DMSO-d^6) δ 7.64 (d, *J* = 7.6 Hz, 2H), 7.45 – 7.38 (m, 3H), 7.32 (t, *J* = 7.3 Hz, 1H), 7.24 (s, 1H), 7.00 (q, *J* = 5.1 Hz, 1H), 6.56 (d, *J* = 8.6 Hz, 1H), 6.53 (s, 1H), 3.86 (s, 2H), 2.72 (d, *J* = 4.9 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d^6) δ 190.74, 175.29, 156.21, 153.53, 137.41, 135.98, 130.68, 129.69, 129.44, 129.38, 127.50, 126.21, 125.90, 32.35, 25.82. HRMS (ESI$^+$) calcd for $C_{17}H_{15}NO$ $[M+H]^+$: 250.1226, found $[M+H]^+$: 250.1203.

Compound **anti-S_3**



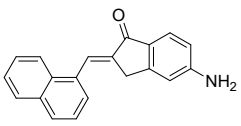
Yellow powder (91.0%). The spectra were consistent with those reported previously.[2] ^{1}H-NMR (700 MHz, DMSO-d^6)δ 7.70 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 8.6 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 1H), 7.32 (d, *J* = 2.4 Hz, 1H), 6.80 – 6.75 (m, 2H), 3.95 (d, *J* = 2.3 Hz, 2H), 3.06 (s, 6H). HRMS (ESI$^+$) calcd for $C_{18}H_{18}NO$ $[M+H]^+$: 264.1388, found $[M+H]^+$: 264.1378.

Compound **anti-S_4**



Yellow powder (59.0%). ^{1}H-NMR (700 MHz, DMSO-d^6) δ 8.27 (s, 1H), 8.05 – 8.01 (m, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 5.2 Hz, 1H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.60 – 7.54 (m, 2H), 7.52 – 7.44 (m, 2H), 6.67 (s, 1H), 6.62 (d, *J* = 8.4 Hz, 1H), 6.43 (s, 2H), 4.03 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d^6) δ 190.63, 156.25, 153.44, 137.81, 133.64, 133.53, 133.28, 130.84, 129.82, 128.94, 128.78, 128.03, 127.55, 127.52, 127.07, 126.38, 126.32, 114.65, 108.55, 32.26. HRMS (ESI$^+$) calcd for $C_{20}H_{15}NO$ $[M+H]^+$: 286.1226, found $[M+H]^+$: 286.1222.

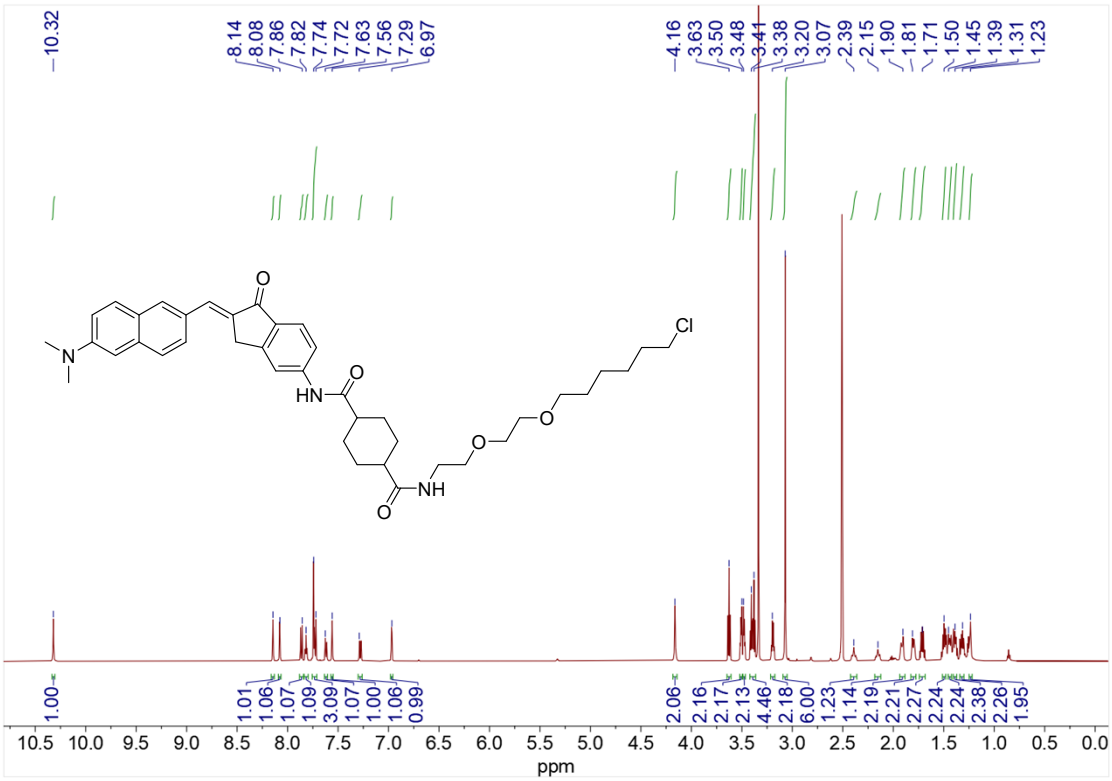
Compound **anti-S_5**



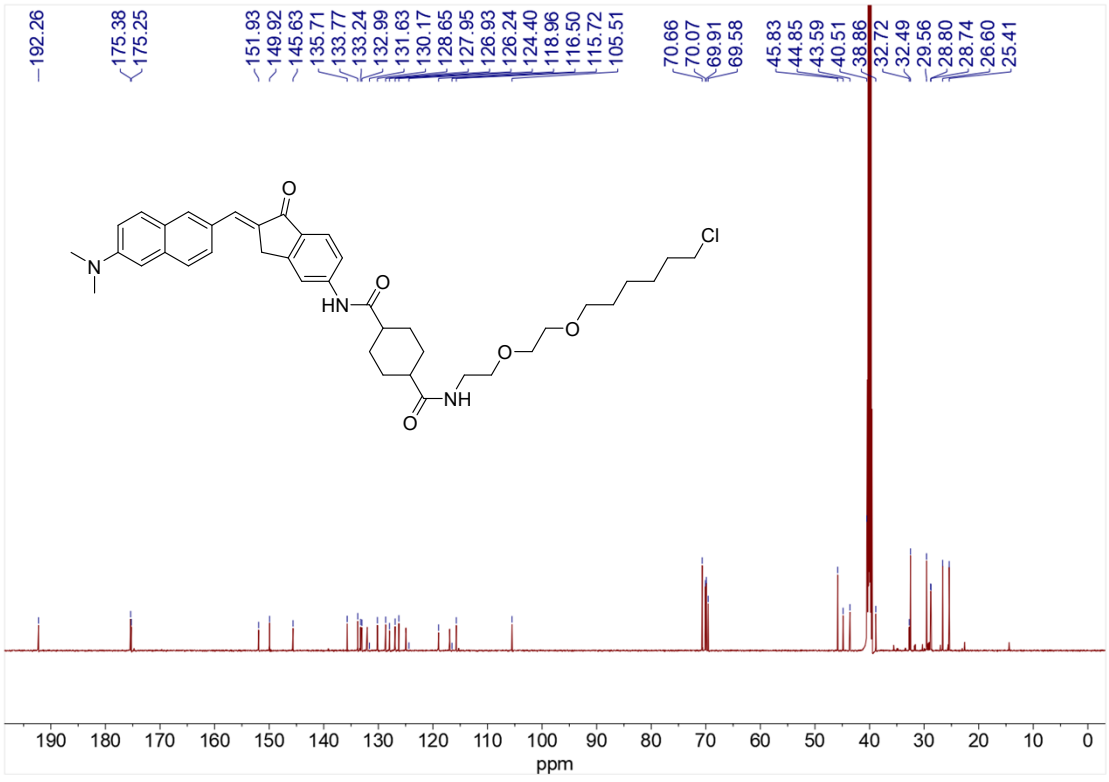
Yellow powder (73.0%). ^{1}H-NMR (700 MHz, DMSO-d^6) δ 8.15 (d, *J* = 8.4 Hz, 1H), 8.03 (s, 1H), 8.00 (dd, *J* = 12.3, 8.2 Hz, 2H), 7.92 (d, *J* = 7.1 Hz, 1H), 7.61 (tt, *J* = 15.7, 7.3 Hz, 3H), 7.52 (d, *J* = 8.4 Hz, 1H), 6.65 – 6.58 (m, 2H), 6.42 (s, 2H), 3.86 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d^6) δ 190.29, 156.28, 153.65, 139.76, 133.78, 132.44, 132.11, 129.60, 129.23, 127.44, 127.34, 126.69, 126.49, 126.46, 126.09, 125.97, 123.90, 114.69, 108.54, 31.78. HRMS (ESI$^+$) calcd for $C_{20}H_{15}NO$ $[M+H]^+$: 286.1226, found $[M+H]^+$: 286.1235.

NMR Spectra

S_4-Halo:

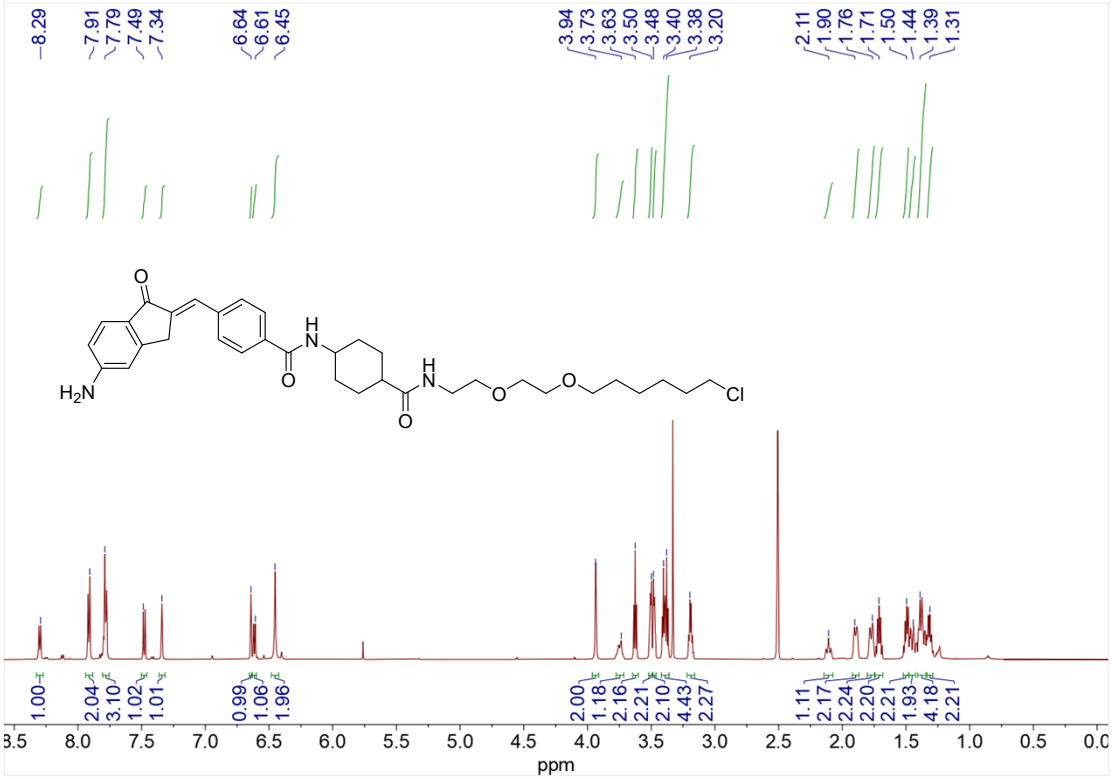


^{1}H-NMR spectrum of **S_4-Halo** (DMSO-d^6)

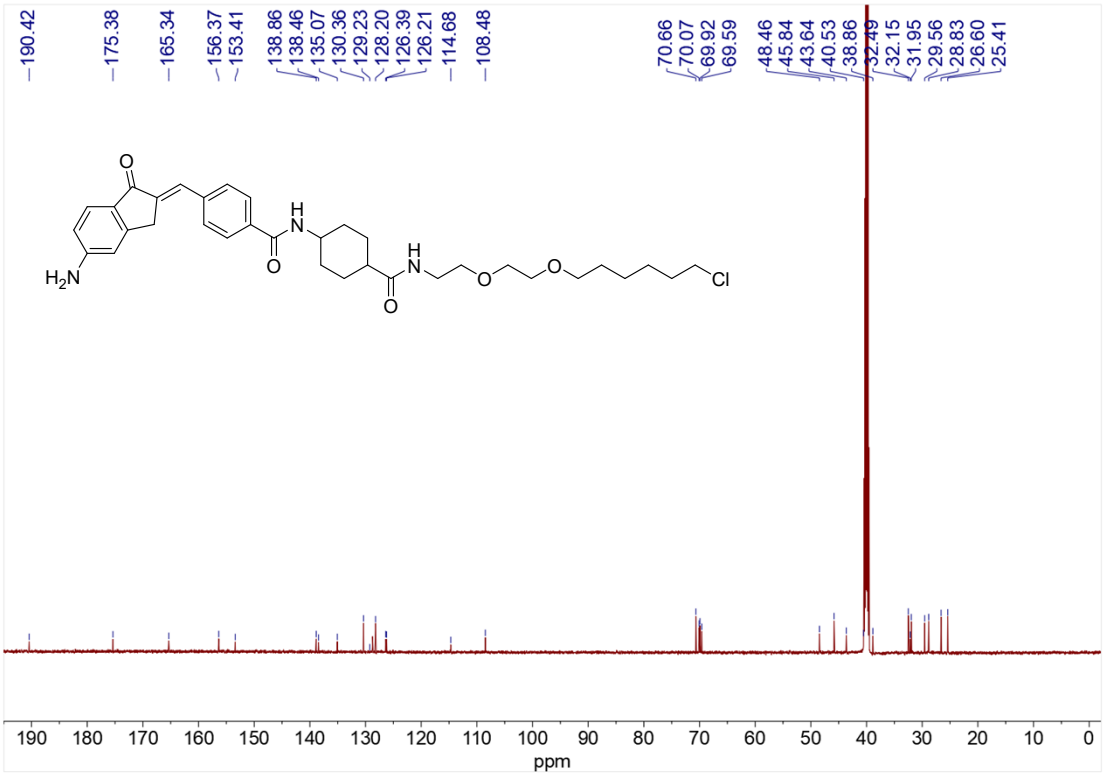


^{13}C-NMR spectrum of **S_4-Halo** (DMSO-d^6)

anti-S_1-Halo:

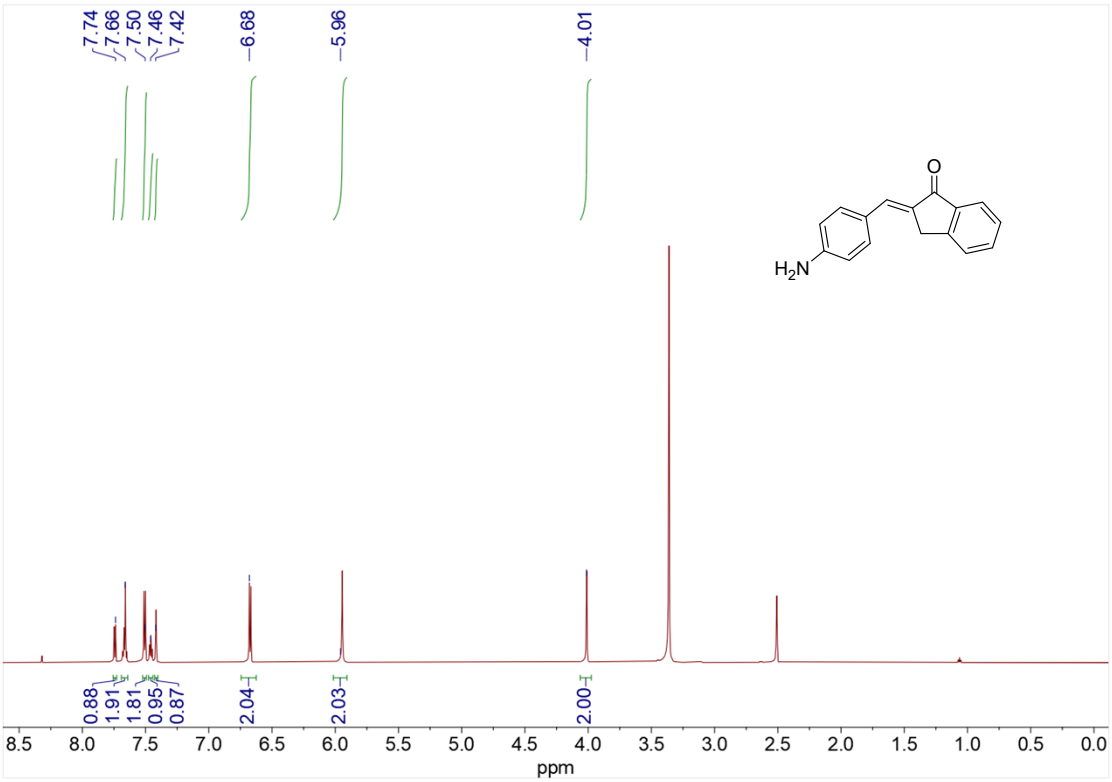


^{1}H-NMR spectrum of **anti-S_1-Halo** (DMSO-d^{6})



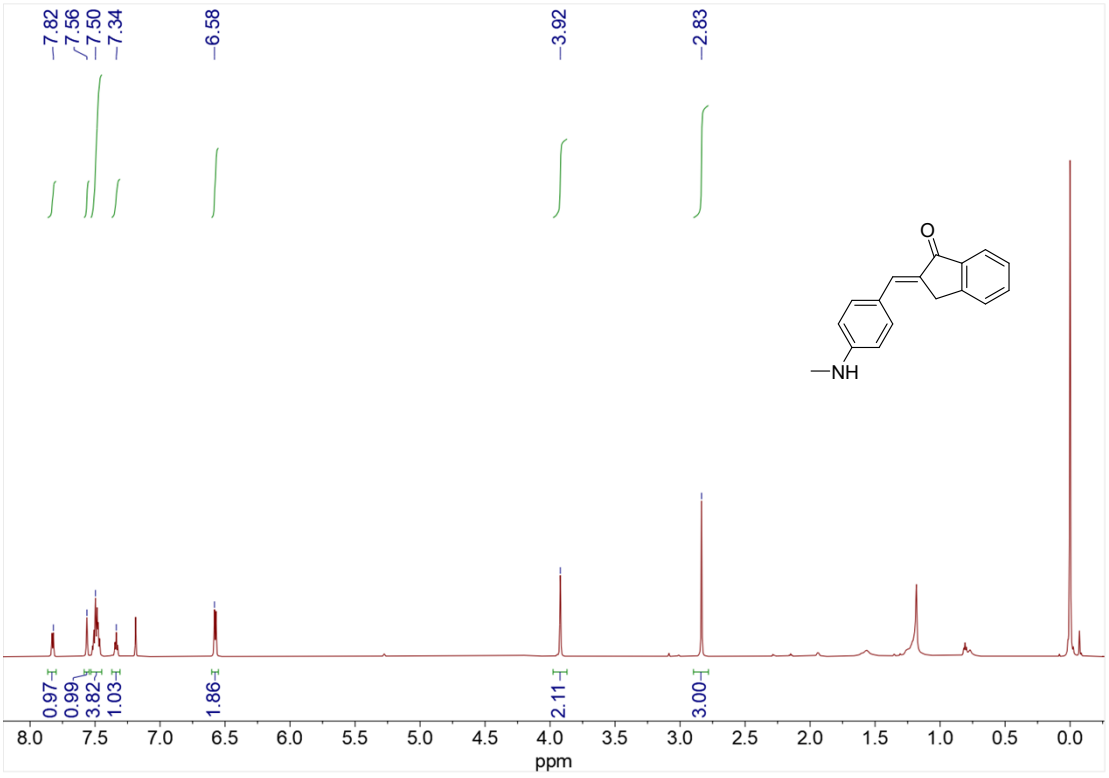
^{13}C-NMR spectrum of **anti-S_1-Halo** (DMSO-d^{6})

S_1:



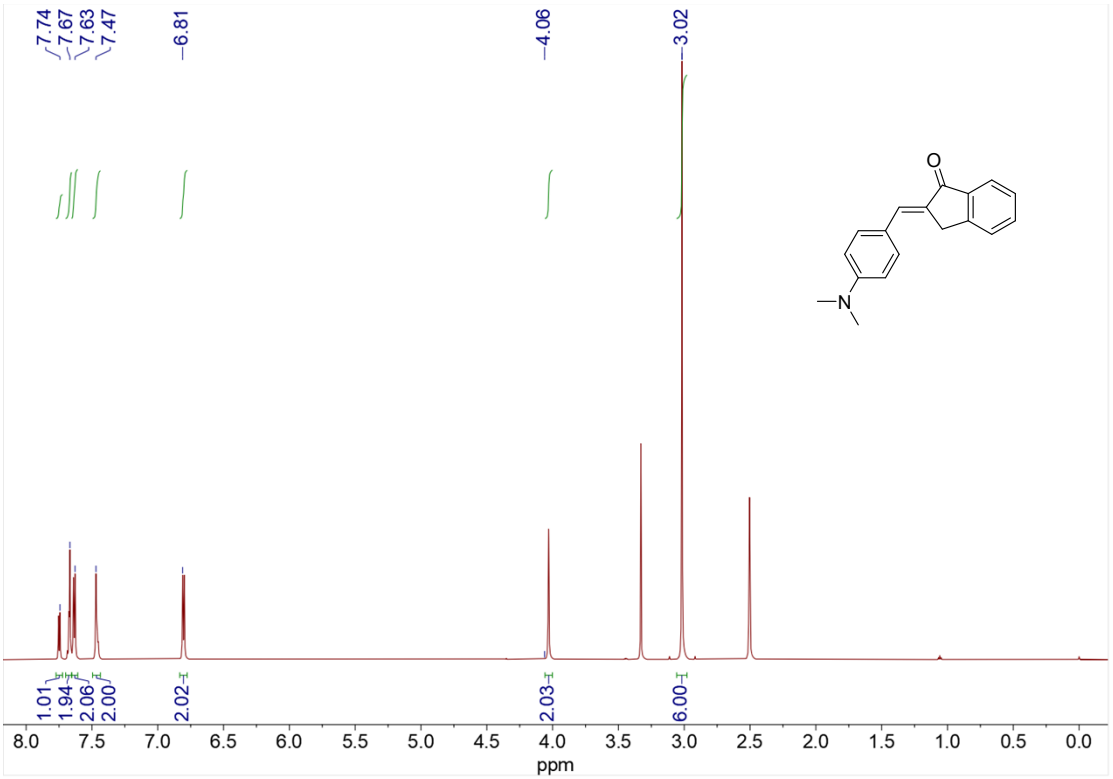
^{1}H-NMR spectrum of **S_1** (DMSO-d^6)

S_2:



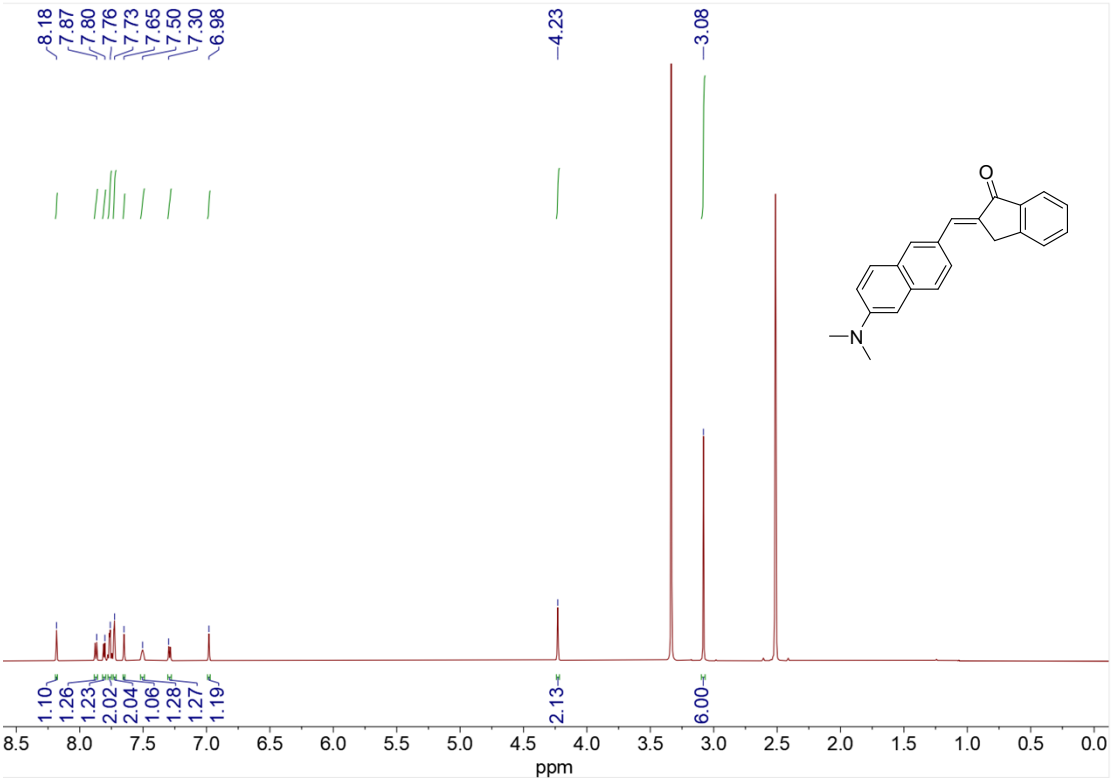
^{1}H-NMR spectrum of **S_2** (Chloroform-d)

S_3:



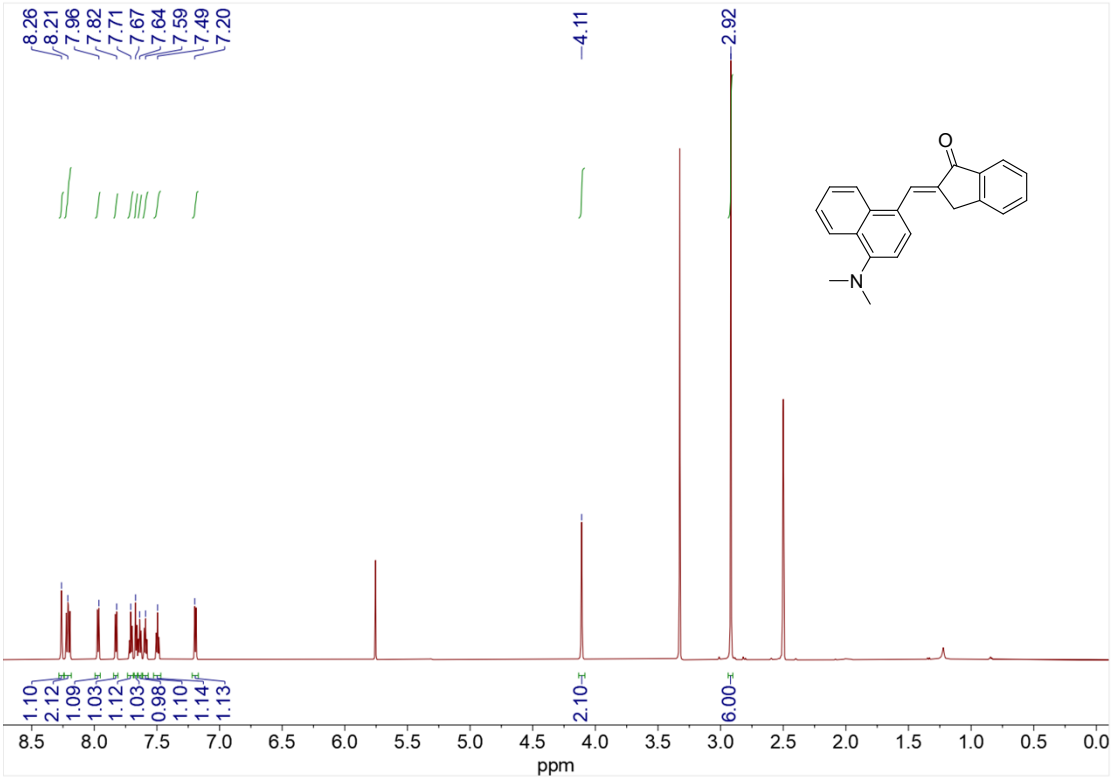
^{1}H-NMR spectrum of **S$_3$** (DMSO-d^{6})

S$_4$:



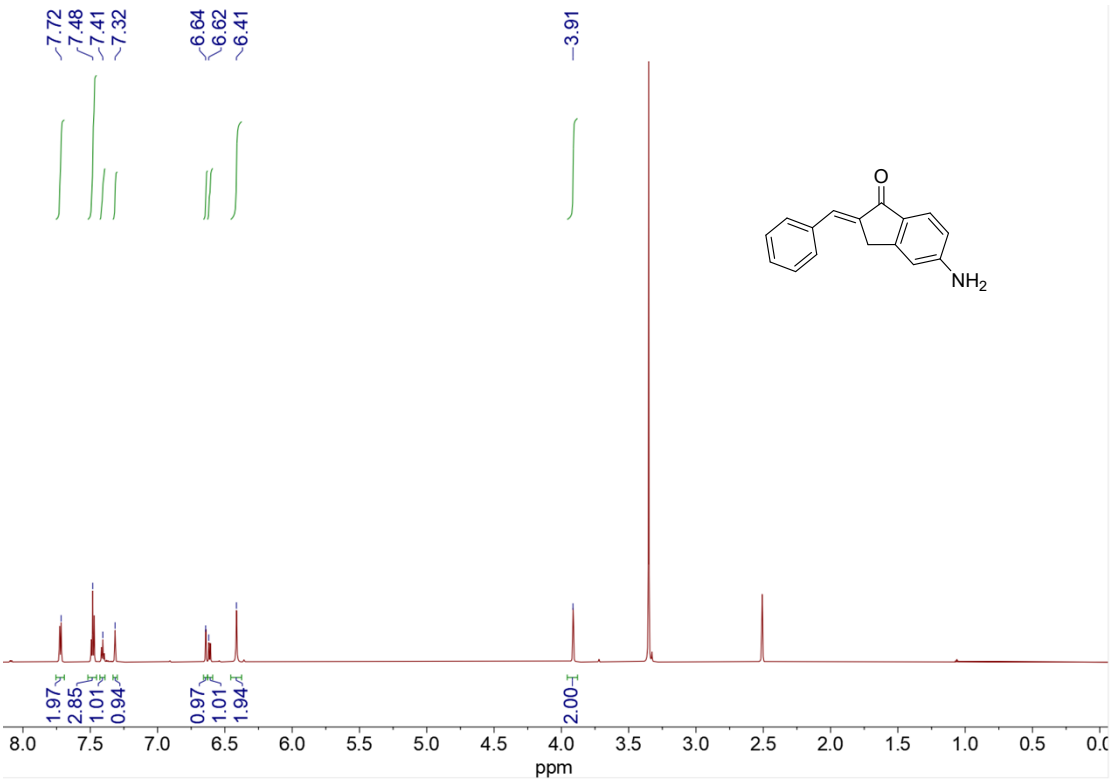
^{1}H-NMR spectrum of **S$_4$** (DMSO-d^{6})

S$_5$:



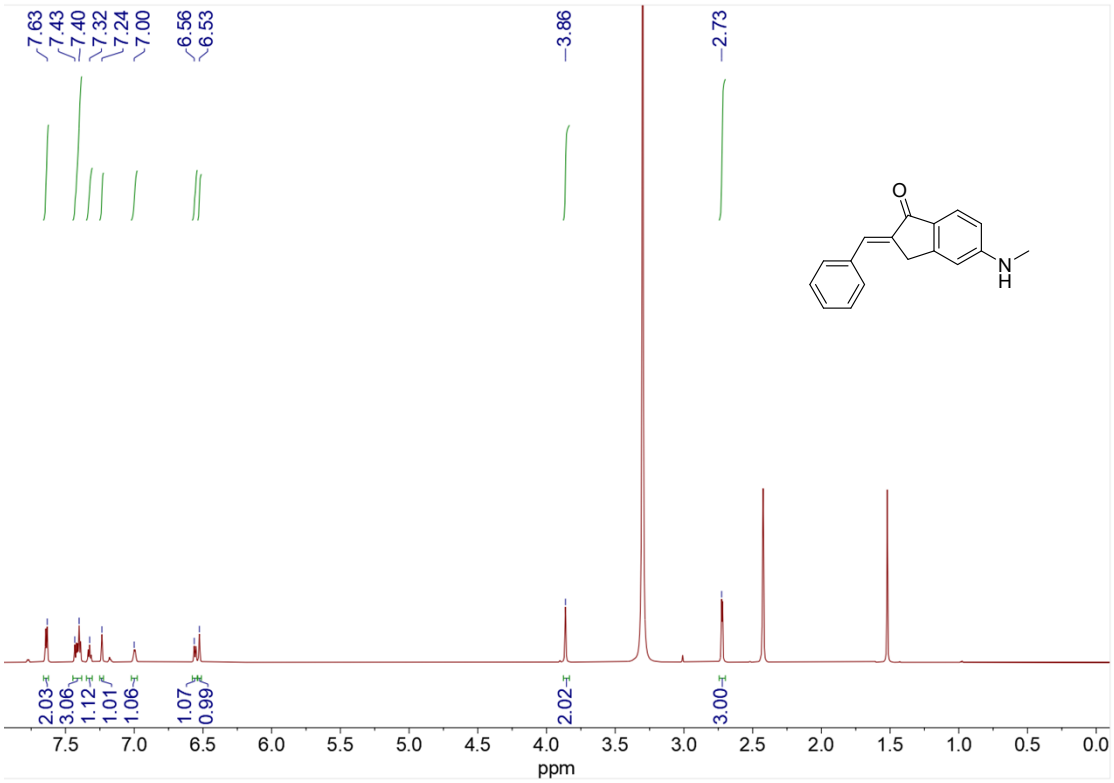
^{1}H-NMR spectrum of **S_5** (DMSO-d^{6})

anti-S_1:



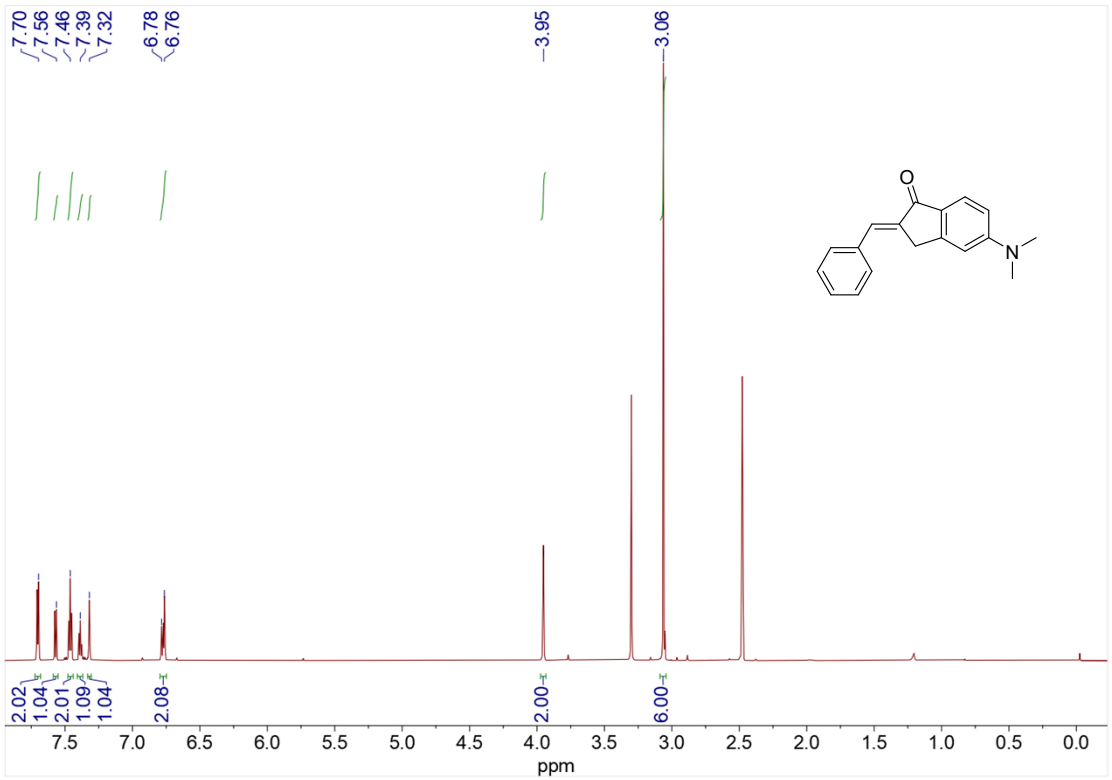
^{1}H-NMR spectrum of **anti-S_1** (DMSO-d^{6})

anti-S_2:



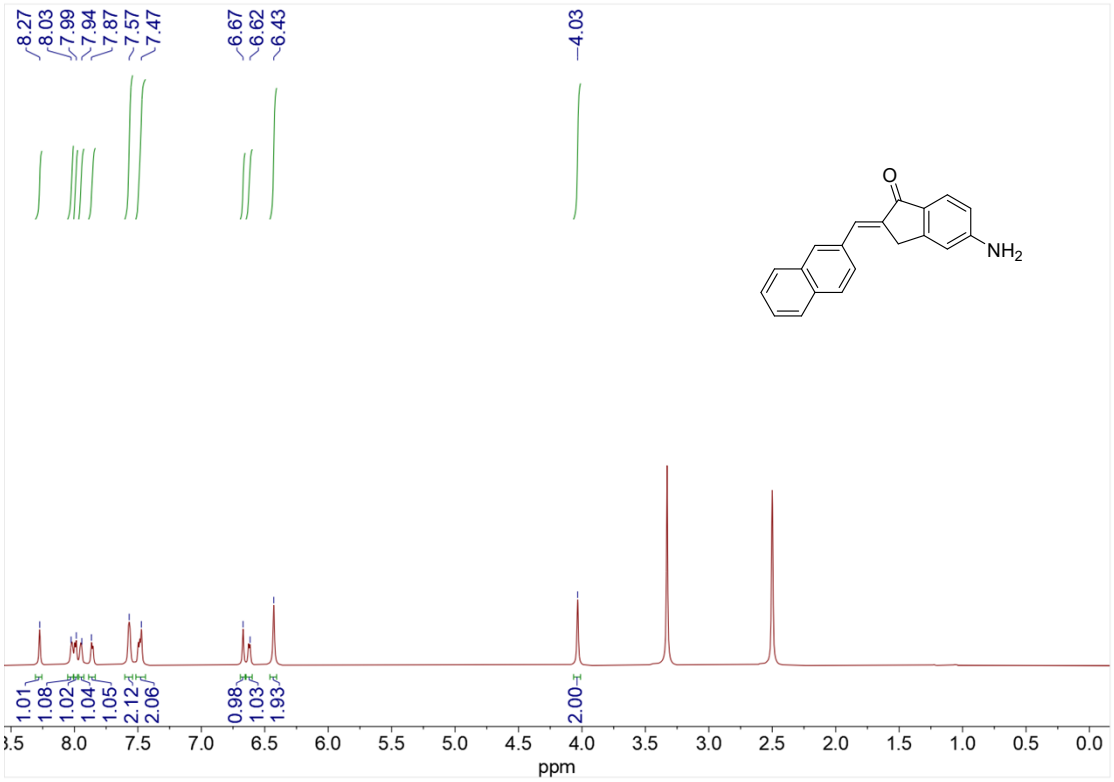
^{1}H-NMR spectrum of **anti-S_2** (DMSO-d^6)

anti-S_3:



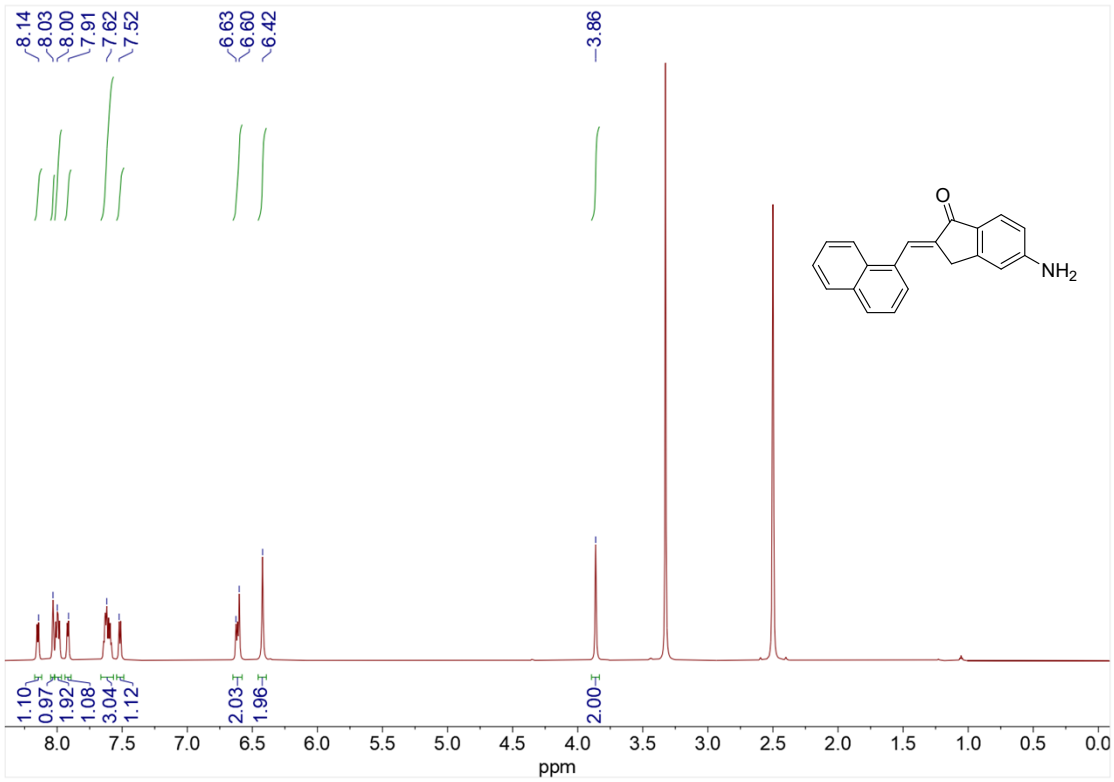
^{1}H-NMR spectrum of **anti-S_3** (DMSO-d^6)

anti-S_4:



^{1}H-NMR spectrum of **anti-S_4** (DMSO-d^6)

anti-S_5:



^{1}H-NMR spectrum of **anti-S_5** (DMSO-d^6)

References

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