Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Selective, Turn-On Fluorescent Probes for Imaging Hydrogen Sulfide in Living Cells

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Materials and Methods

Silica gel (SiliaFlash F60, Silicycle, 230 - 400 mesh) was used for column chromatography. Analytical thin-layer chromatography (TLC) was performed on J. T. Baker silica gel plates (250 μm thickness) and preparative TLC was performed on Silicycle SiliaPlates (1 mm thickness). All compounds subjected to preparative chromatography were protected from light during purification.

4-amino-1,8-naphthalic anhydride, 4-nitro-1,8-naphthalic anhydride, and 2-methoxyethylamine were purchased from Aldrich and used as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories and used as received. Piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES, Aldrich) and potassium chloride (99.999%, Aldrich) were used to make buffered solutions (50 mM PIPES, 100 mM KCl, pH 7.4) with Millipore water. Anhydrous sodium hydrosulfide (NaSH) was purchased from Strem Chemicals and handled under nitrogen. *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) was purchased from Cayman Chemical and stored below -20 ^{o}C prior to use. All other RSONs were purchased from Aldrich. Stock solutions of each RSON were prepared in either buffer or DMSO under nitrogen immediately prior to use and were introduced into buffered solutions with a syringe. Stock solutions of the fluorescent probes were prepared in DMSO and stored in below -20 °C until immediately prior to use.

Spectroscopic Methods

NMR spectra were acquired on either a Varian INOVA 500 or 600 MHz spectrometer at 25.0 ^{o}C. Chemical shifts are reported in parts per million (δ) and are referenced to residual protic solvent resonances. The following abbreviations are used in describing NMR couplings: (s) singlet, (d) doublet, (t) triplet, and (b) broad. IR spectra were measured on a Nicolet Magna 550 FT-IR spectrometer as KBr pellets. Mass spectra were recorded with an Agilent LC/MS mass spectrometer using electrospray ionization in positive ion mode. UV–visible spectra were

acquired on a Cary 100 spectrometer equipped with a Quantum Northwest TLC-42 dual cuvette temperature controller at 25.00 ± 0.05 °C. Fluorescence spectra were obtained on a Quanta Master 40 spectrofluorimeter (Photon Technology International) equipped with a Quantum Northwest TLC-50 temperature controller at either 25.0 ± 0.05 or 37.0 ± 0.05 °C. All fluorescence measurements were made under anaerobic conditions, with cuvette solutions prepared under an inert atmosphere in septum-sealed cuvettes obtained from Starna Scientific. All UV–vis and fluorescence experiments were repeated at least in triplicate.

Cell Culture and Imaging Materials and Methods

HeLa cells were obtained from ATCC and cultured in Dulbecco's Modified Eagle Medium (DMEM, Cellgro, MediaTek, Inc.) supplemented with 10% fetal bovine serum (FBS, HyClone), and 1% penicillin/streptomycin. Prior to imaging, cells were passed and plated into poly-D-lysine coated plates (MatTek) containing 2 mL of DMEM, and incubated at 37 ^{o}C with 5% CO2. For H_2S detection studies, the cells were then co-incubated with the H_2S probe (5 μM), Hoechst 33258 nuclear dye (2.5 μM), and NaSH (250 μM) in DMEM. Prior to imaging, cells were washed with 2 mL of phosphate buffered saline (PBS) and then bathed in 2 mL of PBS during imaging.

Differential interference contrast (DIC) and fluorescent images were obtained using a Nikon Eclipse TE200U inverted epifluorescence microscope equipped with a 40x objective. The microscope was operated with the MetaVue Imaging Series software package. All fluorescent images were corrected for background.

Syntheses

6-bromo-2-(2-methoxyethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3**).

4-bromo-1,8-naphthalic anhydride (1.0 g, 3.6 mmol) and 2-methoxyethylamine (314 µL, 3.61 mmol) were combined in 60 mL of EtOH. The reaction mixture was protected from light, heated to 85 °C, and refluxed for 15 h. The reaction mixture was allowed to cool to room temperature and the ethanol was removed under vacuum to afford the desired product as a brown powder (952 mg, 91% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.58 (d, *J* = 7.2 Hz, 1H, ArH), 8.48 (d, *J* = 9.0 Hz, 1H, ArH), 8.33 (d, *J* = 7.8 Hz, 1H, ArH), 7.96 (d, *J* = 7.8 Hz, 1H, ArH), 7.76 (t, *J* = 7.6 Hz, 1H, ArH), 4.36 (t, *J* = 5.8 Hz, 2H, CH_2), 3.66 (t, *J* = 5.8 Hz, 2H, CH_2), 3.31 (s, 3H, CH_3). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ: 163.71, 163.68, 133.3, 132.2, 131.3, 131.1, 130.6, 130.3, 129.0, 128.0, 123.0, 122.1, 69.5, 58.8, 39.4.

6-amino-2-(2-methoxyethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4**).

4-amino-1,8-naphthalic anhydride (81.7 mg, 383 µmol) and 2-methoxyethylamine (36 µL, 422 µmol) were combined in a vial with 5 mL of EtOH and a stir bar, protected from light, and heated at 80 ^{o}C for 14 h. After cooling to room temperature, the solvent was removed under vacuum, and the product was purified by SiO_2 chromatography (CH_2Cl_2 to 3% MeOH/CH_2Cl_2) to yield the desired amine product (67 mg, 65% yield). R_f (1:1 hex:EtOAc): 0.50. ^{1}H NMR (500 MHz, DMSO): δ 8.69 (d, *J* = 8.0 Hz, 1H, ArH), 8.49 (d, *J* = 7.0 Hz, 1H, ArH), 8.25 (d, *J* = 8.5 Hz, 1H, ArH), 7.72 (t, *J* = 7.8 Hz, 1H, ArH), 7.51 (bs, 2H, NH_2), 6.90 (d, *J* = 8.5 Hz, 1H, ArH), 4.27 (t, *J* = 6.5 Hz, 2H, CH_2), 3.61 (t, *J* = 6.5 Hz, 2H, CH_2), 3.31 (s, 3H, CH_3). ^{13}C{^{1}H} NMR (125 MHz, DMSO): δ 164.3, 163.4, 153.2, 134.5, 131.6, 130.2, 129.9, 124.5, 122.2, 119.8, 108.7, 107.9, 69.3, 58.4, 38.6. Calcd *m/z* for [M + H^+]: 271.11, obs. 270.99.

2-(2-methoxyethyl)-6-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione (**HSN1**).

4-nitro-1,8-naphthalic anhydride (97.6 mg, 401 µmol) and 2-methoxyethylamine (37 µL, 435 µmol) were combined in a vial with 8 mL of EtOH and a stir bar, protected from light, and

heated to 80 °C for 14 h to yield a homogenous reaction mixture. The reaction mixture was cooled to 0 °C to precipitate the product, which was isolated by filtration, washed with cold EtOH, and dried under vacuum (73 mg, 61% yield). R_f (1:1 hex:EtOAc): 0.83. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.85 (d, *J* = 8.0 Hz, 1H, ArH), 8.76 (d, *J* = 7.5 Hz, 1H, ArH), 8.72 (d, *J* = 8.5 Hz, 1H, ArH), 8.42 (d, *J* = 8.5 Hz, 1H, ArH), 8.00 (t, *J* = 8.0 Hz, 1H, ArH), 4.48 (t, *J* = 5.0 Hz, 2H, CH_2), 3.76 (t, *J* = 5.0 Hz, 2H, CH_2), 3.40 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$): δ 164.4, 162.6, 149.6, 132.6, 130.0, 129.9, 129.3, 129.2, 126.9, 123.9, 123.7, 123.0, 69.4, 58.8, 39.7. IR (KBr, cm^{-1}): 3074 (w), 2903 (w), 1703 (s), 1660 (s), 1582 (m, sh), 1527 (s, *v*(NO_2)), 1456 (w), 1408 (m), 1376 (s), 1333 (s, *v*(NO_2)), 1230 (s), 1181 (w), 1111 (s), 1062 (m), 1010 (s), 786 (s), 760 (s).

6-azido-2-(2-methoxyethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**HSN2**).

6-bromo-2-(2-methoxyethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3**) (100 mg, 299 μmol) and sodium azide (35.2 mg, 539 μmol) were dissolved in 1-methyl-2-pyrrolidinone (3 mL). The reaction mixture was protected from light and heated to 110 °C for 3 h. After cooling to room temperature, the black/brown solution was diluted with 25 mL of H_2O. The crude product was extracted with ethyl acetate (4 x 25 mL) and dried over $MgSO_4$. The product was purified by preparative TLC in the dark using 5% Methanol in CH_2Cl_2 as the eluent to give the desired product as a pale yellow solid (17.7 mg, 36% yield). R_f (1:1 hex:EtOAc): 0.82. ^{1}H NMR (600 MHz, DMSO) δ: 8.63 (d, *J* = 7.3 Hz, 1H, ArH), 8.57 (d, *J* = 7.9 Hz, 1H, ArH), 8.42 (d, *J* = 8.5 Hz, 1H, ArH), 7.72 (t, *J* = 7.9 Hz, 1H, ArH), 7.45 (d, *J* = 7.9 Hz, 1H, ArH), 4.42 (t, *J* = 6.5 Hz, 2H, CH_2), 3.72 (t, *J* = 6.2 Hz, 2H, CH_2), 3.37 (s, 3H, CH_3). $^{13}C\{^1H\}$ NMR (150 MHz, DMSO) δ: 163.7, 163.2, 143.3, 132.1, 131.2, 128.8, 128.7, 127.7, 123.9, 122.4, 118.4, 116.3, 69.0, 58.4, 39.0. Calcd *m/z* for [M^+]: 296.09, obs. 296.14. IR (KBr, cm^{-1}): 2930 (w), 2828 (w), 2128 (s, *v*(N_3)), 1694 (s), 1666 (s, sh), 1588 (s), 1578 (s), 1515 (w), 1418 (m), 1379 (m), 1349 (s), 1293 (s), 1239 (m), 1093 (m), 781 (m), 753 (w).

Selectivity Data

	Fluorescence Enhancement (F/F_o)					
Time	**H_2S**	**Blank**	**Cys**	**GSH**	**ALA**	**H_2O_2**
0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
1	1.6 ± 0.5	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.2
5	4 ± 1	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.2
10	7 ± 2	1.2 ± 0.2	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.2
15	9 ± 2	1.2 ± 0.2	1.3 ± 0.1	1.2 ± 0.12	1.2 ± 0.1	1.3 ± 0.2
30	11 ± 1	1.1 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	1.3 ± 0.1	1.4 ± 0.2
45	13 ± 2	1.1 ± 0.1	1.6 ± 0.2	1.7 ± 0.4	1.4 ± 0.1	1.4 ± 0.2
60	14 ± 2	1.1 ± 0.1	1.7 ± 0.2	1.9 ± 0.5	1.4 ± 0.1	1.5 ± 0.2
75	14 ± 2	1.1 ± 0.1	1.8 ± 0.2	2.1 ± 0.6	1.5 ± 0.1	1.5 ± 0.2
90	15 ± 2	1.1 ± 0.1	1.8 ± 0.2	2.3 ± 0.7	1.5 ± 0.1	1.6 ± 0.2

	Fluorescence Enhancement (F/F_o)				
Time	**NO**	**Na_2SO_3**	**$Na_2S_2O_3$**	**Cys***	**GSH***
0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
1	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.6 ± 0.4	1.2 ± 0.1
5	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.8 ± 0.5	1.6 ± 0.4
10	1.1 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	2.3 ± 0.6	2.3 ± 0.9
15	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	2.6 ± 0.7	3 ± 1
30	1.2 ± 0.2	1.4 ± 0.1	1.1 ± 0.1	2.9 ± 0.7	5.7 ± 0.6
45	1.2 ± 0.2	1.5 ± 0.1	1.1 ± 0.1	4 ± 1	7.6 ± 0.7
60	1.2 ± 0.2	1.5 ± 0.1	1.2 ± 0.1	5 ± 1	10 ± 1
75	1.2 ± 0.2	1.6 ± 0.2	1.2 ± 0.1	6 ± 2	10.1 ± 0.6
90	1.2 ± 0.2	1.6 ± 0.2	1.2 ± 0.1	6 ± 2	12 ± 1

Table S1. Selectivity of **HSN1** with other reactive oxygen, nitrogen, and sulfur species. (5 μM probe, 100 equiv. of RSONS, 50 mM PIPES, 100 mM KCl, pH 7.4, 37 °C, λ_{ex} = 435 nm, λ_{scan} = 450 – 750 nm). Data were acquired before analyte addition and 1, 5, 10, 15, 30, 45, 60, 75, and 90 min after analyte addition. Comparisons of 2,000 equiv. (10 mM) of cysteine and GSH are shown in the lower right of the table (denoted Cys* and GSH*). The data shown are the average of at least three runs.

	Fluorescence Enhancement (F/F_o)					
Time	**H_2S**	**Blank**	**Cys**	**GSH**	**ALA**	**H_2O_2**
0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
1	9 ± 1	1.5 ± 0.1	2.2 ± 0.2	2.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1
5	25 ± 3	2.0 ± 0.2	3.5 ± 0.5	4.6 ± 0.2	2.3 ± 0.1	2.6 ± 0.3
10	39 ± 7	2.3 ± 0.4	4 ± 1	6.1 ± 0.5	2.6 ± 0.1	3.1 ± 0.4
15	47 ± 5	2.6 ± 0.5	5 ± 1	7 ± 1	3.0 ± 0.2	3.4 ± 0.5
30	55 ± 8	2.9 ± 0.6	6 ± 1	9 ± 1	3.5 ± 0.4	3.6 ± 0.6
45	59 ± 8	3.0 ± 0.7	7 ± 2	10 ± 1	4.2 ± 0.7	3.9 ± 0.7
60	61 ± 9	3.2 ± 0.7	8 ± 2	11 ± 2	5 ± 1	4.0 ± 0.7

	Fluorescence Enhancement (F/F_o)				
Time	**NO**	**Na_2SO_3**	**$Na_2S_2O_3$**	**Cys***	**GSH***
0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
1	1.2 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1
5	1.3 ± 0.1	2.2 ± 0.1	1.8 ± 0.2	3.5 ± 0.1	4.2 ± 0.3
10	1.3 ± 0.1	2.8 ± 0.2	2.0 ± 0.2	4.0 ± 0.1	5.1 ± 0.5
15	1.3 ± 0.1	3.2 ± 0.3	2.2 ± 0.2	4.6 ± 0.1	5.9 ± 0.6
30	1.3 ± 0.1	4.2 ± 0.4	2.3 ± 0.2	5.6 ± 0.2	7.1 ± 0.8
45	1.4 ± 0.1	4.7 ± 0.5	2.4 ± 0.3	6.5 ± 0.2	8.3 ± 0.8
60	1.4 ± 0.1	5.1 ± 0.5	2.5 ± 0.3	7.5 ± 0.3	9.4 ± 0.9

Table S2. Selectivity of **HSN2** with other reactive oxygen, nitrogen, and sulfur species. (5 μM probe, 100 equiv. of RSONS, 50 mM PIPES, 100 mM KCl, pH 7.4, 37 °C, λ_{ex} = 435 nm, λ_{scan} = 450 – 750 nm). Data were acquired before analyte addition and 1, 5, 10, 15, 30, 45, and 60 min after analyte addition. Comparisons of 2,000 equiv. (10 mM) of cysteine and GSH are shown in the lower right of the table (denoted Cys* and GSH*). The data shown are the average of at least three runs.

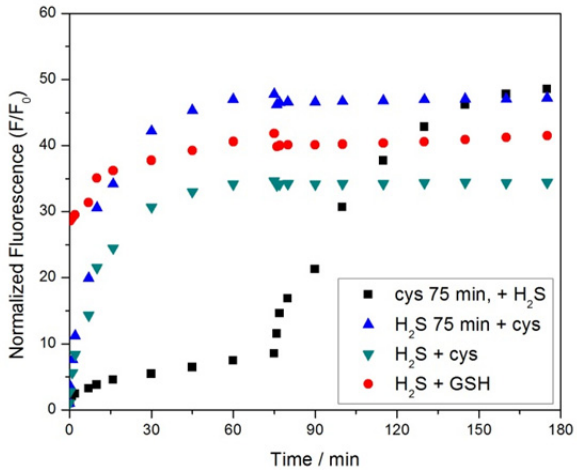

Figure S1. Reaction of H_2S with **HSN2** in the presence of cysteine or GSH does not appreciably affect the ability of **HSN2** to detect H_2S. (5 μM probe, 100 equiv. of RSONs, 50 mM PIPES, 100 mM KCl, pH 7.4, 37 °C, λ_{ex} = 435 nm, λ_{scan} = 450 – 750 nm). (■) incubated with cysteine for 75 min, followed by addition of H_2S. (▲) incubated with H_2S for 75 min, followed by addition of cysteine. (▼) H_2S and cysteine added at the same time. (●) H_2S and GSH added at the same time.

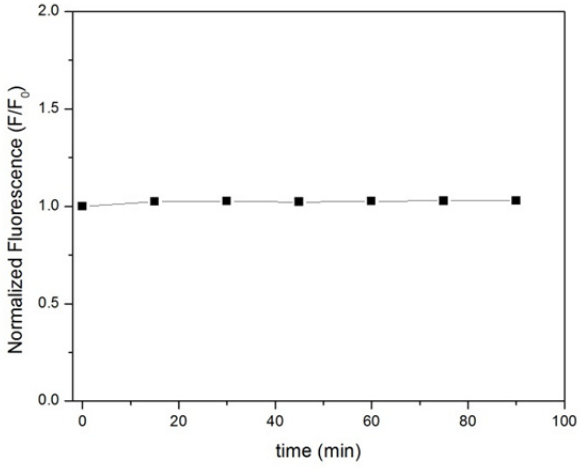

Figure S2. Photostability of isolated **4**. (5 μM **4**, 50 mM PIPES, 100 mM KCl, pH 7.4, 37 °C, λ_{ex} = 435 nm, λ_{scan} = 450 – 750 nm).

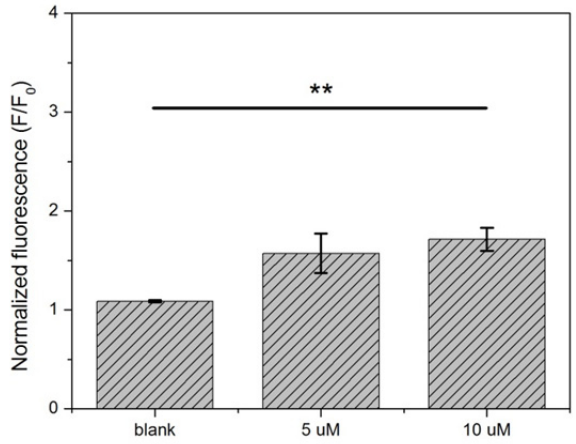

Figure S3. Detection limit of **HSN1** after 60 min incubation with H_2S. (5 μM probe, 50 mM PIPES, 100 mM KCl, pH 7.4, 37 °C, λ_{ex} = 435 nm, λ_{scan} = 450 – 750 nm). Each data point reports the mean ± S.E. obtained from three independent experiments, ** indicates $p < 0.01$ (ANOVA).

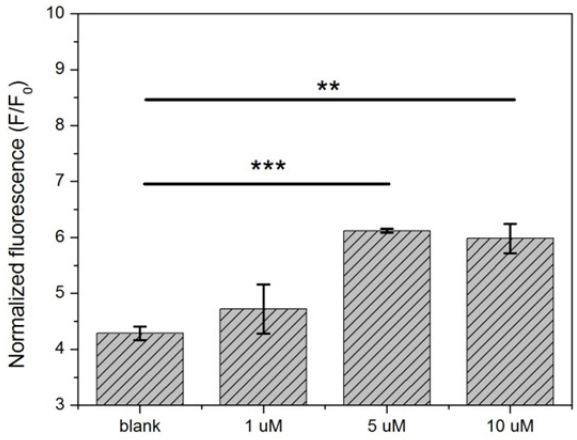

Figure S4. Detection limit of **HSN2** after 60 min incubation with H_2S. (5 μM probe, 50 mM PIPES, 100 mM KCl, pH 7.4, 37 °C, λ_{ex} = 435 nm, λ_{scan} = 450 – 750 nm). Each data point reports the mean ± S.E. obtained from three independent experiments, ** indicates $p < 0.01$, *** indicates $p < 0.001$ (ANOVA).

NMR Spectra

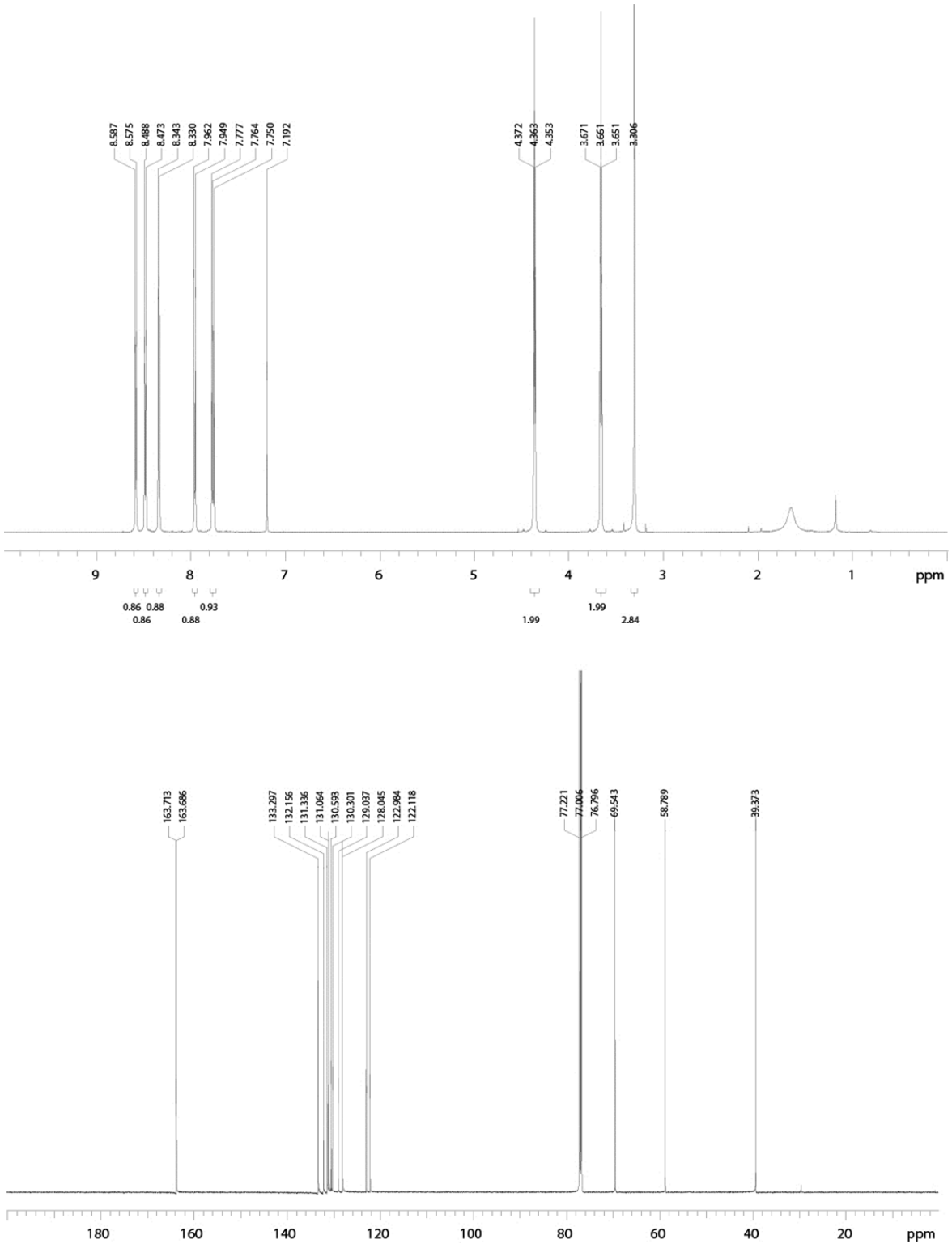

Figure S5. ^{1}H (600 MHz, $CDCl_3$) and $^{13}C\{^{1}H\}$ (150 MHz, $CDCl_3$) NMR spectra of **3**.

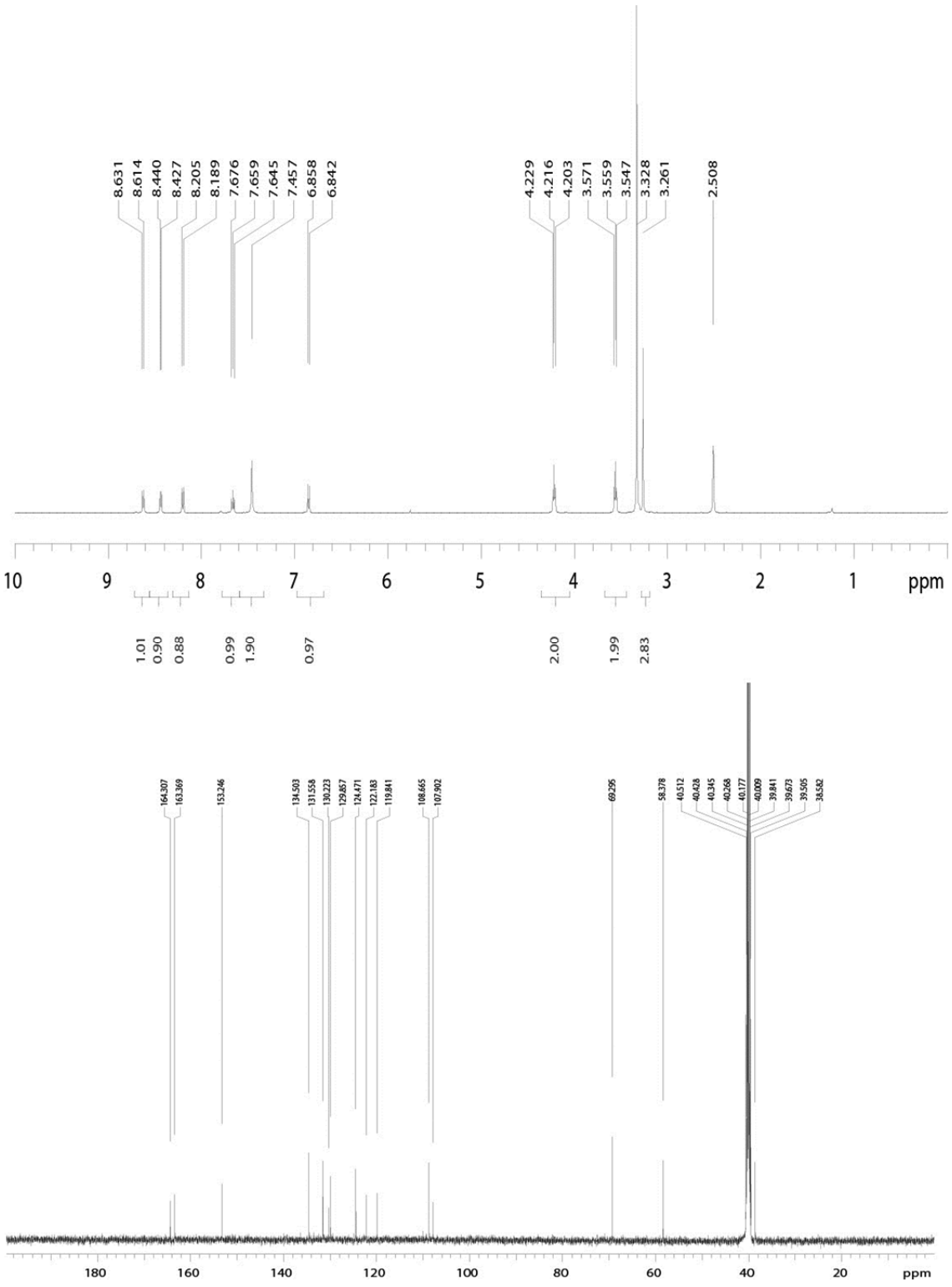

Figure S6. ^{1}H (500 MHz, DMSO) and $^{13}C\{^{1}H\}$ (125 MHz, DMSO) NMR spectra of **4**.

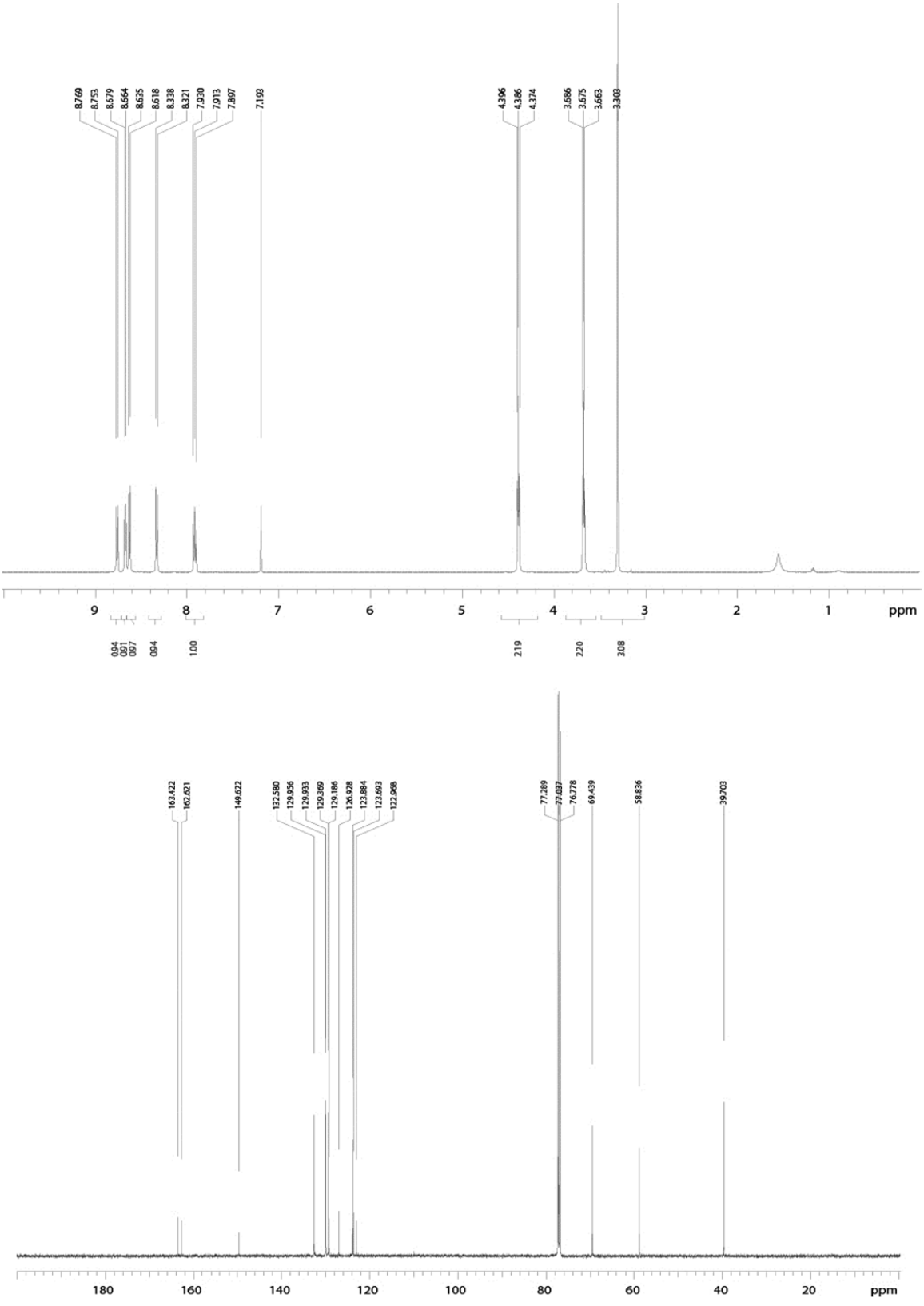

Figure S7. ^{1}H (500 MHz, $CDCl_3$) and $^{13}C\{^{1}H\}$ (125 MHz, $CDCl_3$) NMR spectra of **HSN1**.

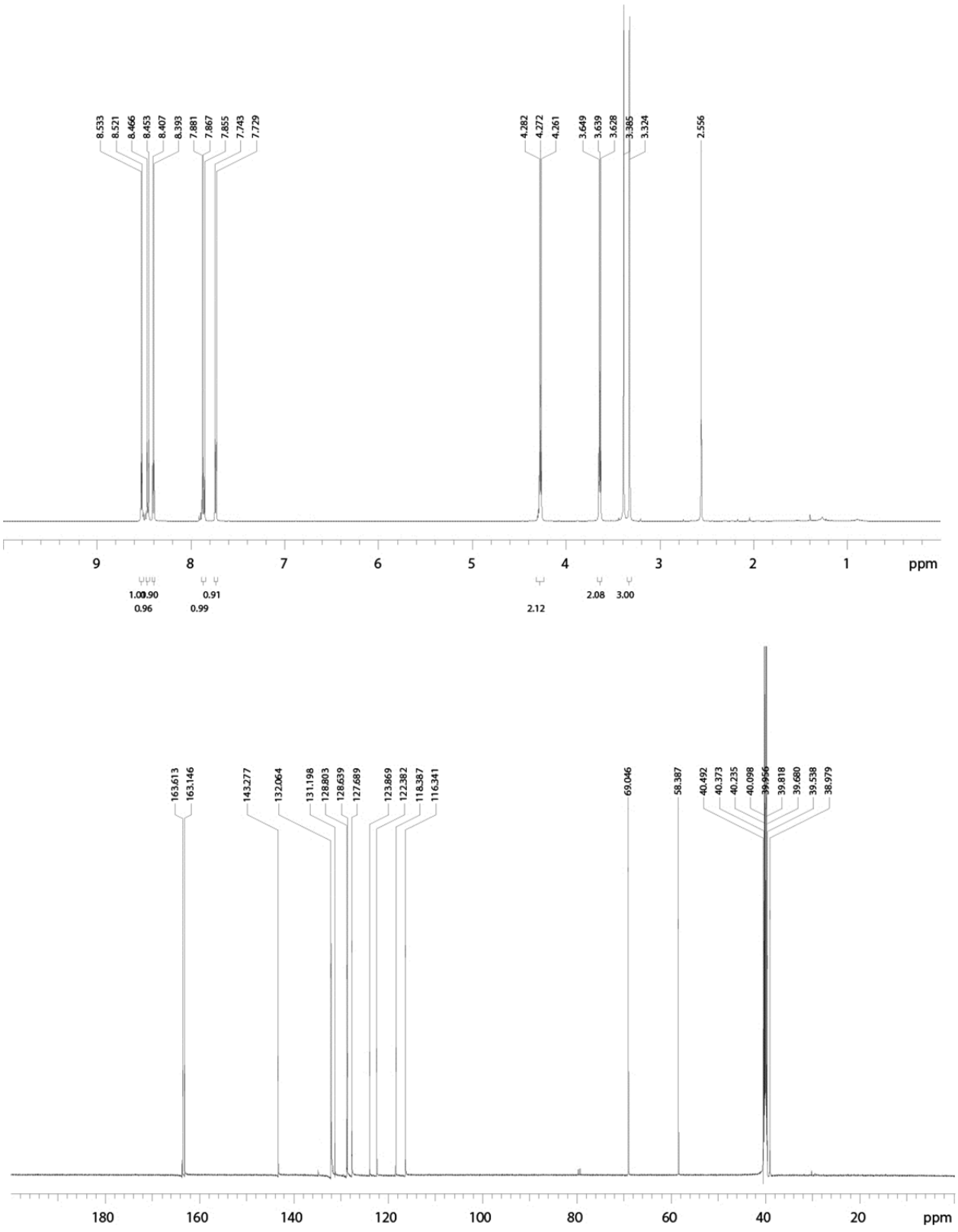

Figure S8. ^{1}H (600 MHz, DMSO) and $^{13}C\{^{1}H\}$ (150 MHz, DMSO) NMR spectra of **HSN2**.