Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Geminal-Dithiol-based Precursors for Reactive Sulfur Species

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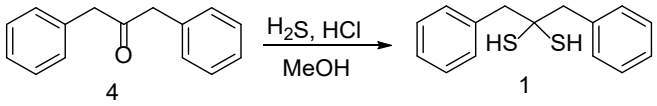
Materials and Equipment

Reagents and solvents were of the highest grade available. Hydrogen sulfide(g) was purchased from Sigma-Aldrich (295442-227G). Chemical reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.062 mm). ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 600 MHz and are reported in parts per million (ppm) on the δ scale relative to $CDCl_3$ (δ 7.26 for ^{1}H, δ 77.16 for ^{13}C), DMSO-d_6 (δ 2.50 for ^{1}H, δ 39.52 for ^{13}C), CD_3CN (δ 1.94 for ^{1}H). UV spectra were measured on a Thermo Evolution 350 UV spectrometer (Thermo USA). Fluorescence spectra were measured on a Cary Eclipse fluorescence spectrophotometer (Agilent, USA). H2S-specific electrode assays were conducted using a monometer and SULF-NP probe (Unisense, Demark). Fluorescent cell imaging was performed on a BZX-800 fluorescent microscope (Keyence, Japan). 5/6

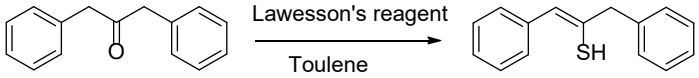
Synthesis of compound 1



4 1

Under 0 ºC, H_2S and HCl gas were simultaneously bubbled into a stirred solution of **4** (5.6 g, 26 mmol) in anhydrous methanol (20 ml). A pink color was observed after 30 min. The bubbling was continued for 2 hours. The reaction was then allowed to warm to room temperature and stirred for another 5 hours. The formation of solid precipitate was observed. The precipitate was then collected by filtration and recrystallized in pentane at 40 ºC to afford **1** (4.5 g, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 7.27 (m, 10H), 3.27 (s, 4H), 2.41 (s, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 136.0, 131.5, 127.9, 127.3, 56.9, 53.0.

Synthesis of compound 5/6



4 **6**

A suspension of **4** (2.1 g, 10 mmol) and Lawesson's reagent (4.84 g, 12 mmol) in toluene was heated to reflux overnight. Solvent was removed and the residue was subjected to flash column chromatography to obtain **6** (1g, 44% yield). Characterization matches reported data.[1]

H_2S Generation from 1 measured by the H_2S trapping method

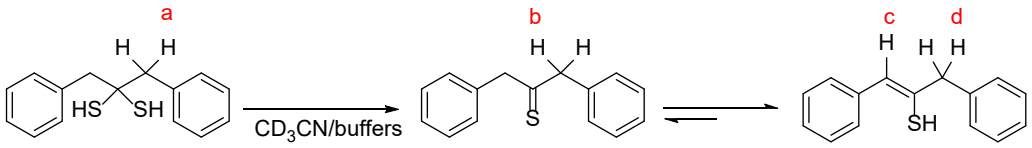
The stock solution of **1** (15 mM) was freshly prepared in acetonitrile. 500 µl of **1** stock solution was added to 4.5 ml pH 5, 6, 7.4, 8 buffers to make 1.5 mM of **1**. These solutions were put into 20 ml scintillation vials. To each vial, an Eppendorf tube containing 500 µl 10% zinc acetate and a filter paper (1 cm x 2cm) was placed. The vials were then capped and sealed with parafilm and incubated at room temperature for 3 hours. The contents in the Eppendorf vials were transferred to 4 ml vials, and $FeCl_3$ (1 mL, 30 mM in 1.2M HCl) and N,N-dimethyl-1,4-phenylenediamine sulfate (1 mL, 20 mM in 7.2M HCl) were added. The mixture was further incubated at room temperature for 15 min. 500 µl of such solution was then taken and diluted with 3 mL PBS buffer

(100 mM, pH 7.4). The UV absorbance of the solution was measured at 670 nm. Experiments were done in triplicates.

H_2S Generation from 1 measured by H_2S-selective electrode

The stock solution of **1** (38.4 mM) was freshly prepared in acetonitrile. 10.4 µl of **1** stock solution was added to sealed scintillation vials containing 7.92 ml pH 5, 6, 7, 8 buffers and 70 µl acetonitrile, which makes 50 µM **1** in 8 ml 1% acetonitrile/buffers. H_2S release from the resulting solutions was monitored by a H_2S electrode (SULF-NP, Unisense A/S, Inc. Denmark). H_2S concentrations were determined using standard curves with Na_2S under each pH. Experiments were done in triplicates.

NMR study of the decomposition of compound 1



15 mg **1** was dissolved in 0.5 mL CD_3CN. 0.1 mL deuterated buffer (pD 7.8, equivalent of pH 7.4) or D_2O was then added. NMR was taken periodically to monitor the decomposition of **1**.

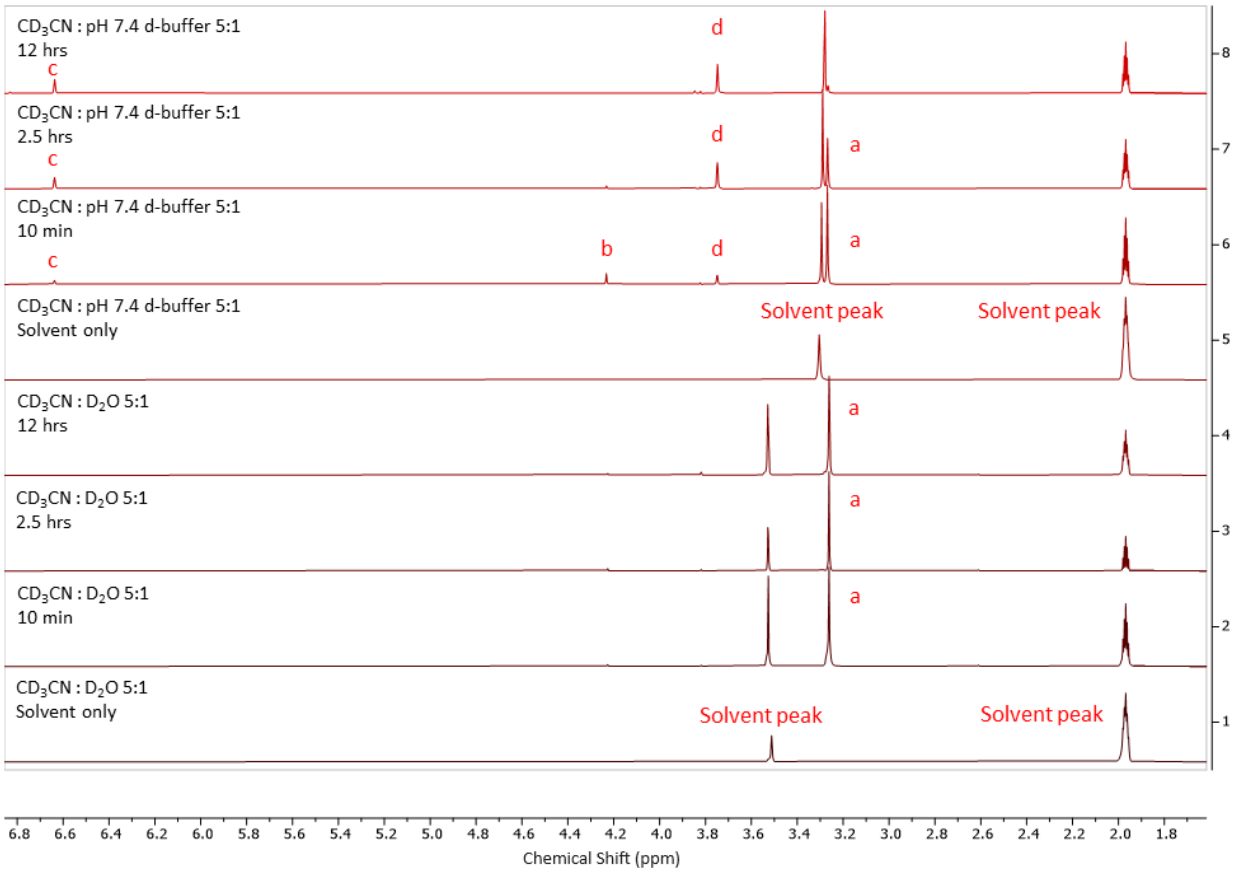


Figure S1. H-NMR spectra of the decomposition of compound **1**.

H_2S Generation from 6 measured by the H_2S trapping method

The stock solution of **6** (100 mM) was freshly prepared in THF. The stock solution of Na_2S was freshly prepared in H_2O. 500 µL of **6** stock solution was added to 4.5 mL of the following solutions to make 10 mM solutions: a) pH 7.4 PBS buffer; b) 20% Hela cell lysate in buffer; c) 10% bovine plasma in buffer; d) 20 mM cysteine in buffer. As a positive control, Na_2S (5 mM) in PBS buffer was made. These solutions were put into 20 ml scintillation vials. To each vial, an Eppendorf tube containing 1 mL 10% zinc acetate and a filter paper (1 cm x 2cm) was placed. The vials were then capped and sealed with parafilm and incubated at room temperature for 12 hours. The contents in the Eppendorf vials were transferred to 4 mL vials, and $FeCl_3$ (1 mL, 30 mM in 1.2M HCl) and N,N-dimethyl-1,4-phenylenediamine sulfate (1 mL, 20 mM in 7.2M HCl) were added. The mixture was further incubated at room temperature for 15 min. 500 µL of such solution was then taken and diluted with 3 mL PBS buffer (100 mM, pH 7.4). UV absorbance of the resulting solution was measured at 670 nm. Experiments were done in triplicates.

Generation of 4 from 1 or 6 in the presence of buffers

1 or **6** (50 mg) was incubated in 20 mL 1:1 CH_3CN/PBS buffer (100 mM, pH 8) for overnight. The mixture was then extracted by DCM. The organic layer was washed with water and brine, and dried over $MgSO_4$. After filtration and concentration, the crude material was subjected to flash column chromatography to provide ketone **4** (6.8 mg, 17% yield for **1**, 8.3 mg, 18% yield for **6**).

In-situ generation of compound 2

1 (50 mg, 0.19 mmol) was dissolved in $CHCl_3$ (3 mL). The solution was cooled to -20 °C, followed by the addition of 90% t-butyl nitrite (26 µl, 0.19 mmol). The mixture was stirred at -20 °C for 5 min before the solvent and byproducts were rapidly removed by rotavapor. **2** was redissolved in corresponding solvents and used immediately.

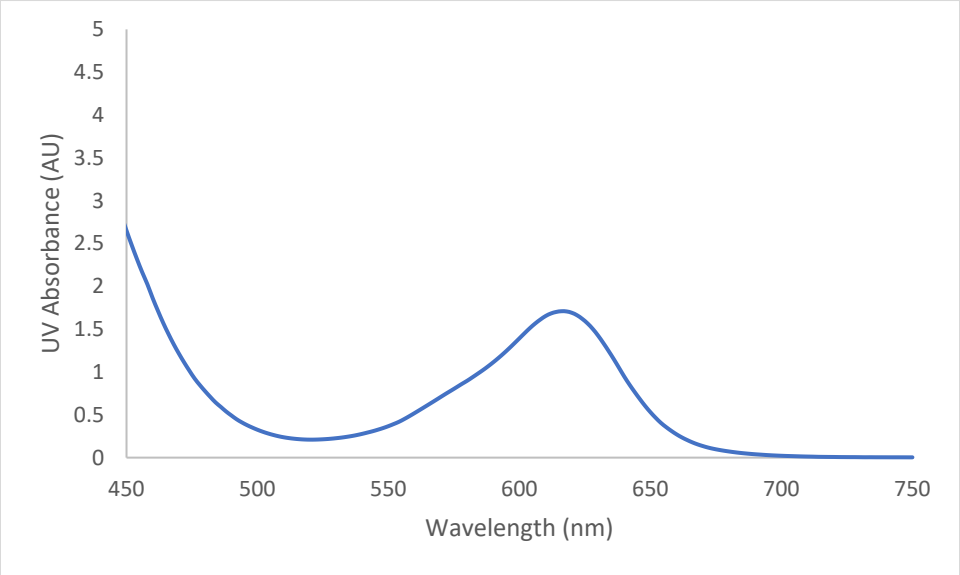


Figure S2. UV-vis absorbance of **2** (64 mM in $CHCl_3$).

NO Generation from 2 measured by gas trapping method with NO Red

2 was in-situ generated by the method described above. A stock solution of **2** (64 mM) in THF was then prepared. 400 µL of **2** stock solution was added to 3.6 mL PBS buffers (pH 5, 6, 7.4, 8)

to make 6.4 mM of **2**. As the positive control, a solution of the pyrrolidine-based NONOate (6.4 mM in 10% THF/pH 7.4 PBS buffer) was prepared. These solutions were put into 20 ml scintillation vials. To each vial, an Eppendorf tube containing a filter paper and NO Red (40 μM in 1 mL acetonitrile and 0.2 mL pH 7.4 PBS buffer) were placed. The vials were then capped and sealed with parafilm and incubated at room temperature for 1.5 hours. The contents in the Eppendorf vials were then transferred to 4 mL vials, and acetonitrile (3 mL) was added. The fluorescence emission spectrum of the mixture was then measured at 593 nm with the excitation at 540 nm. Experiments were done in triplicates.

Thiyl radical generation from 2 measured by TEMPO-9AC

2 was in-situ generated by the method described above. A stock solution of **2** (6.4 mM) in THF was then prepared. TEMPO-9AC, Trityl SNO, and GSNO were dissolved in THF to make their stock solutions of 10 mM. All SNO stock solutions were stored in dark. In a vial, the corresponding TEMPO-9AC and SNO stock solution, THF, and PBS buffer were mixed to make a solution of TEMPO-9AC (20 μM) and SNO (200 μM) in 10% THF/PBS 7.4 buffer. As the negative control, a solution of pyrrolidine-based NONOate (200 μM) with TEMPO-9AC (20 μM) in 10% THF/PBS buffer was also prepared. The solutions were incubated at room temperature for 20 min. The fluorescence emission spectrum of the mixture was then measured at 432 nm with excitation at 361 nm. Experiments were done in triplicates.

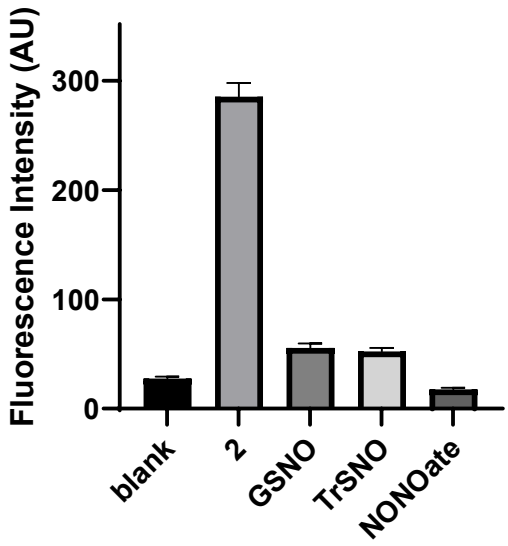


Figure S3. Thiyl radical generation of various species (200 μM) measured by TEMPO-9AC (20 μM). Samples were incubated in 10% THF/PBS 7.4 buffer for 20 min. Fluorescence at 432 nm was measured.

The generation and characterization of 3

To a stirred solution of **1** (50 mg) in $CDCl_3$ (3 ml) was added 90% t-butyl nitrite (2 eq, 51 μL). The green solution was left at rt until the color turned yellow (~2 hours). After confirming the full conversion to **3** by NMR, the reaction was concentrated under reduced pressure to provide the neat form of **3**. The product should be used in the following studies immediately.

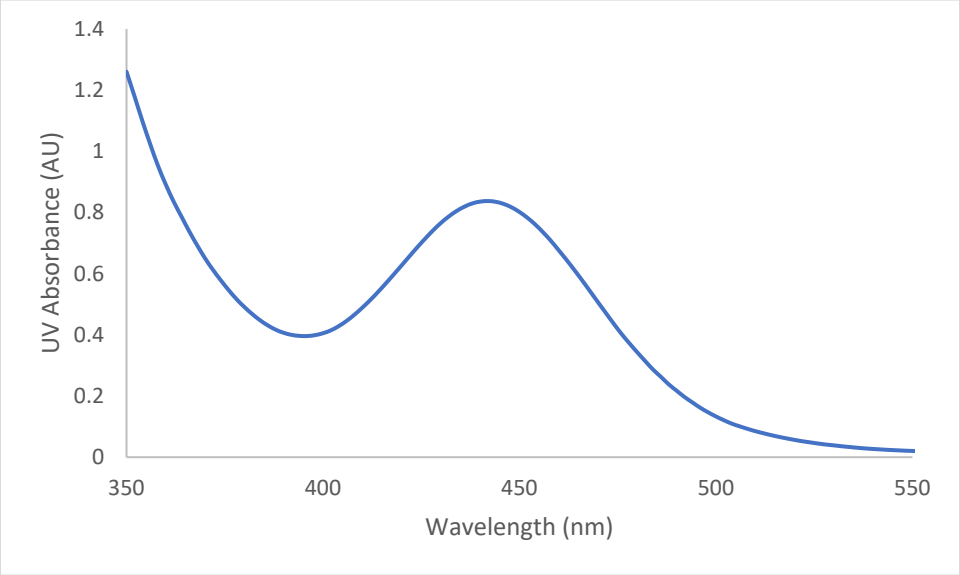


Figure S4. UV-vis absorbance of **3** (10 mM in $CHCl_3$).

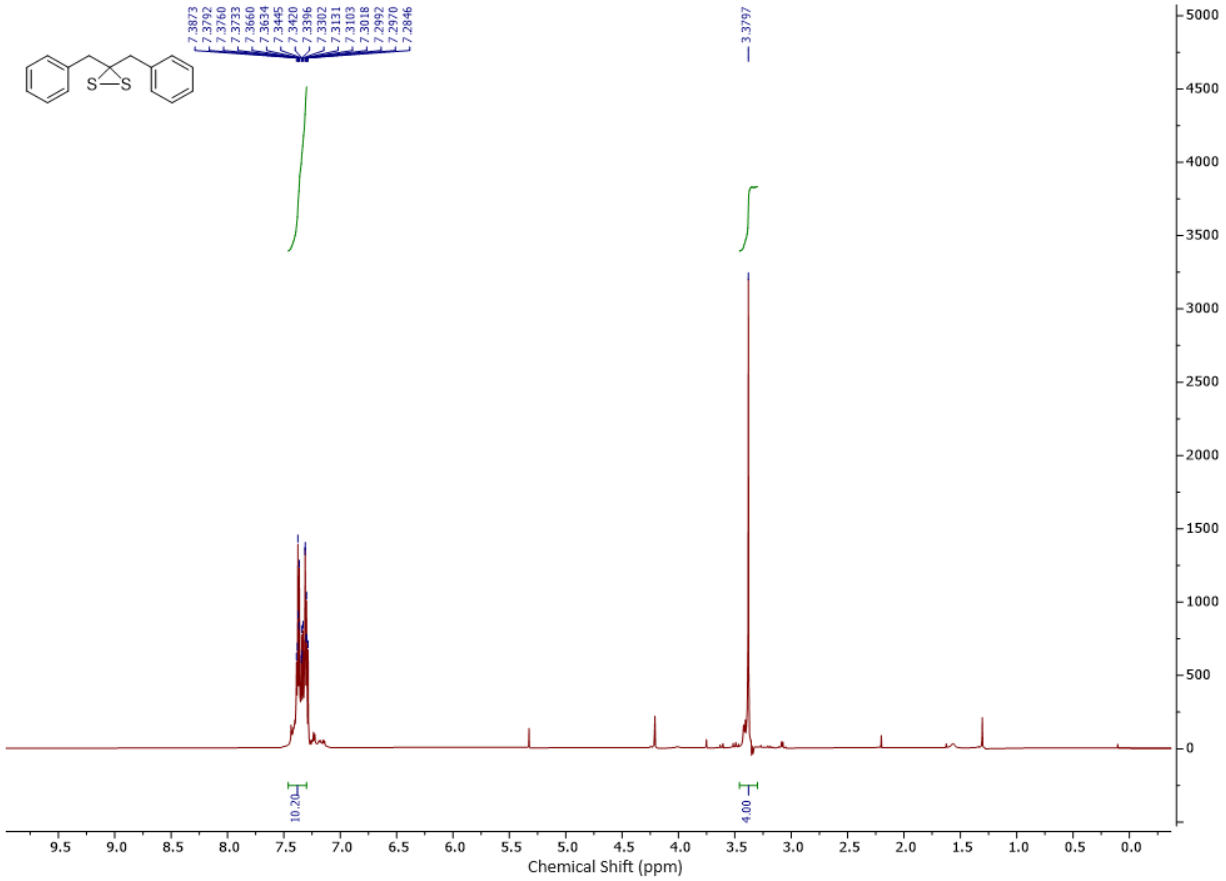


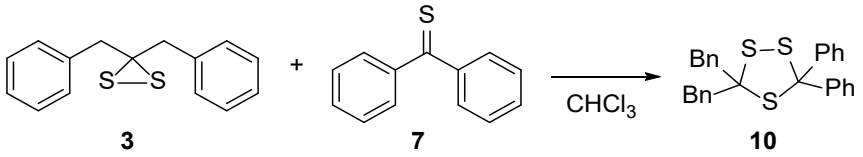
Figure S5. ^{1}H-NMR of **3** (600 MHz in $CDCl_3$).

Reaction of 3 with PPh_3

To a solution of **3** (50 mg, 0.19 mmol) in $CDCl_3$ (3 ml) was added triphenylphosphine (50 mg, 0.19 mmol). The reaction turned pink immediately. Crude NMR showed the formation of **5**/**6**. The

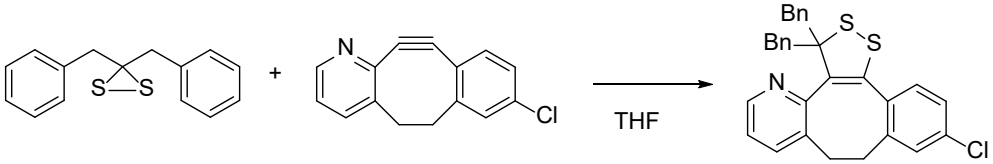
reaction was subjected to flash column chromatography to obtain **5** (20 mg, 45% yield) and S=PPh$_3$ (40 mg, 70% yield). ^{1}H, ^{13}C and ^{31}P NMR of the products matched reported data.

1,3-dipolar cycloadditions of 3



3 **7** **10**

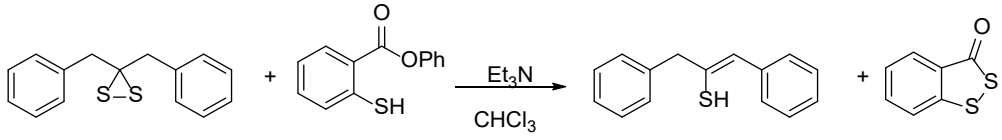
To a solution of **3** (50 mg, 0.19 mmol) in $CHCl_3$ (3 ml) was added a solution of thiobenzophenone **7** (76 mg, 0.37 mmol) in $CHCl_3$ (1 ml). The reaction was allowed to stir for 24 h. The mixture was then concentrated and subjected to preparative TLC to afford **10** (yellow oil, 19 mg, 23% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.46 (m, 4H), 7.38 – 7.34 (m, 4H), 7.33 – 7.29 (m, 6H), 7.26 – 7.22 (m, 6H), 3.57 (d, J = 14.3 Hz, 2H), 3.41 (d, J = 14.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ141.9, 136.8, 131.3, 129.1, 127.9, 127.8, 127.7, 127.0, 91.2, 86.4, 46.5; HRMS calcd for $C_{28}H_{28}NS_3$ $[M+NH_4]^+$ 474.1384, found 474.1365.



3 **11** **12**

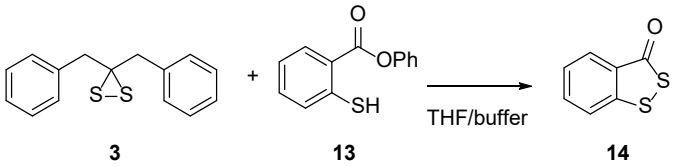
To a solution of **3** (30 mg, 0.11 mmol) in THF (3 mL) was added a solution of **11** (24 mg, 0.1 mmol) in THF (1 mL). The reaction was allowed to stir overnight. TLC showed total consumption of **11**. The mixture was then concentrated and subjected to flash column chromatography to obtain **12** as a white solid (25 mg, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.53 – 7.46 (m, 4H), 7.37 – 7.32 (m, 6H), 7.31 – 7.25 (m, 1H), 7.11 – 6.93 (m, 4H), 3.48 (s, 4H), 2.75 (t, J = 7.4 Hz, 2H), 2.46 (t, J = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.5, 146.7, 141.1, 138.9, 136.7, 133.6, 133.5, 132.0, 131.3, 130.0, 129.2, 128.5, 128.0, 127.1, 126.1, 122.4, 70.1, 49.3, 34.2, 32.3; HRMS calcd $C_{30}H_{25}ClNS_2$ $[M+H]^+$ 498.1117, found 498.1093.

Trapping persulfide formation from 3 using 13



3 **13** **6** **14**

To a solution of **3** (50 mg, 0.19 mmol) in $CHCl_3$ (2 ml) was added a solution of **13** (44 mg, 0.19 mmol) in $CHCl_3$ (1 mL) and 50 µL triethylamine. The mixture was stirred at rt for 3 hours. The mixture was then directly loaded on silica gel column and subjected to flash column chromatography to provide **6** (14 mg, 32% yield) and **14** (22 mg, 69% yield).



3 **13** **14**

To a solution of **3** (50 mg, 0.19 mmol) in 1:1 THF/PBS 7.4 (4 ml) was added **13** (45 mg, 0.19 mmol) in one portion. The reaction was stirred at rt for 5 hours. The mixture was then extracted with DCM. The organic layer was washed with water and brine. The solution was dried over $MgSO_4$, concentrated, and subjected to flash column chromatography to provide **14** (23 mg, 74% yield).

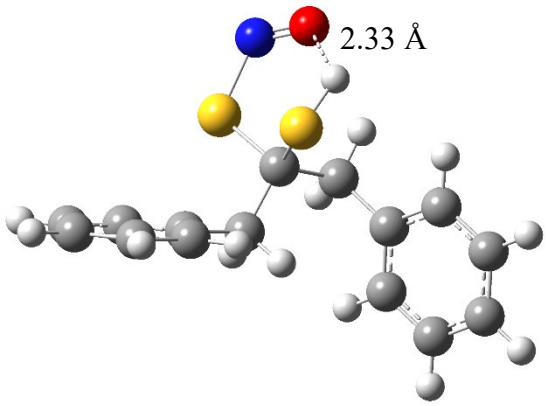
Papain persulfidation induced by 3

Papain was persulfidated using the reported procedure[1] with some modifications. The reduction of the papain solution with cysteine was repeated twice to obtain sufficient protein for the persulfidation treatment steps. Protein-containing fractions pooled from a PD-10 column were quantified by Nanodrop. The two highest concentration fractions from the separate papain reduction steps were then combined and quantified by Nanodrop (242.19 μM). From this, 500 μL of reduced papain was incubated with DTNB (50 μM, 4 mM) at rt in the dark for 20 min, purified with a PD-10 column, and quantified by Nanodrop. The highest concentration fraction (104 μM, 500 μL) was incubated with Na_2S solution (8 equiv., stock prepared in MilliQ H_2O) in Tris-HCl (1M, pH 7.4) for 30 min at rt in the dark. At the same time, the reduced papain (242.19 μM, 580.96 μL) was treated with dithiirane **3** (64 mM in THF, 19.04 μL) (8 equiv.) or the reduced papain was diluted in Tris-HCl to 80.7 μM (600 μL total) then treated with thioketone **5** (64 mM in ACN, 6.19 μL) (8 equiv.) for 30 min at rt in the dark. The dilution was necessary due to the lack of sufficient volume of the reduced protein. After purifying and quantifying all treated proteins with the PD-10 columns and Nanodrop, the level of persulfidation on papain was validated by SSP4 following established protocol.[2]

Computational methods

Gas phase geometry optimization was performed with the Gaussian 09 program[3] using B3LYP functional and 6-31++G(d,p) basis set. Single point energies of located minima were performed at the B3LYP/6-311++G(d,p) level of theory, including the PCM solvation model for chloroform. Frequency calculations were performed to confirm stationary points as minima. NBO (natural bond orbital) calculation have been carried out using NBO 3.1 program[4] as implemented in the Gaussian 09 software package. Coordinates and total energies (Hartree) are provided below.

There is a weak H-bonding interaction observed between oxygen lone pair electrons n(O) $\rightarrow\sigma^*$(S-H) with stabilization energy 0.71 kcal/mol.



Charge = 0 Multiplicity = 1

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Sum of electronic and thermal Energies= -1506.665347

Sum of electronic and thermal Enthalpies= -1506.664403

Sum of electronic and thermal Free Energies= -1506.731164

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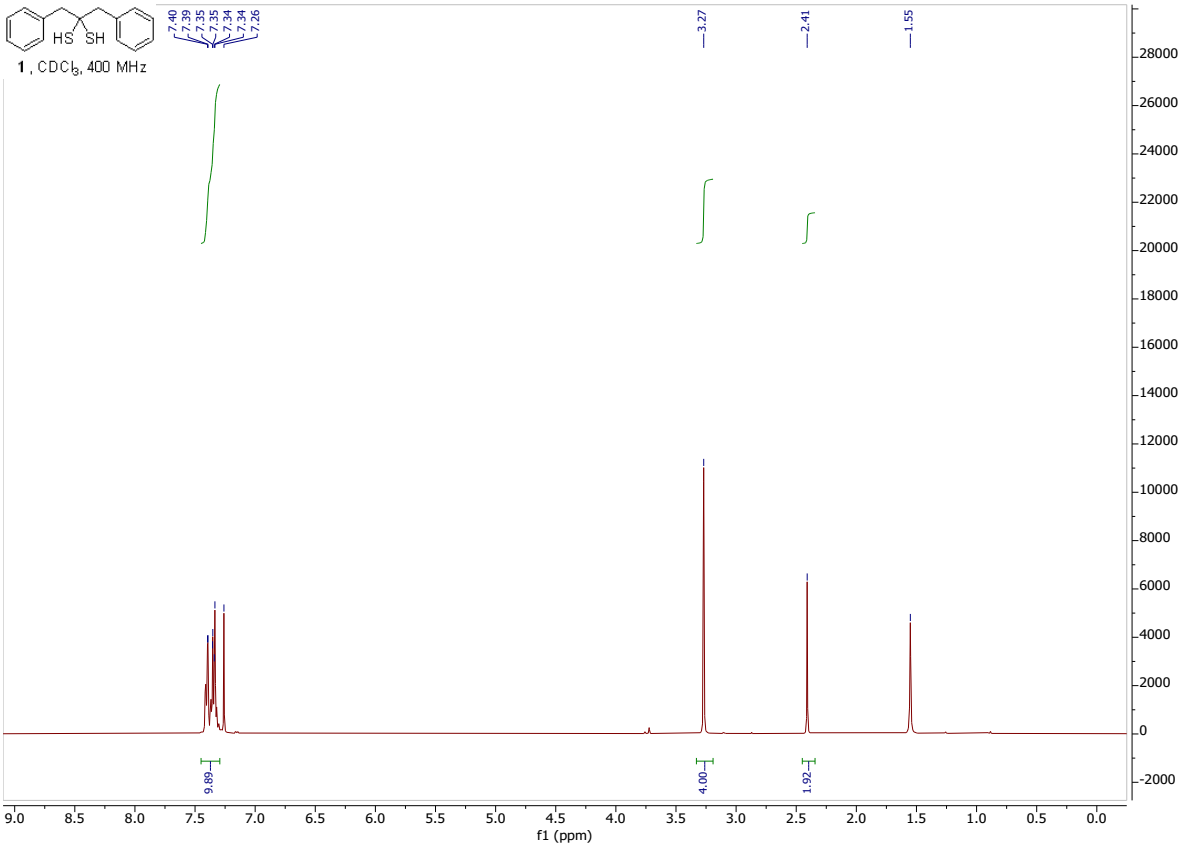
3. *Gaussian 09, Revision D.01*, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, Jr., J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.;

Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; and Fox, D. J. Gaussian, Inc., Wallingford CT, **2013**.
4. Glendening, E. D.; Reed, A. E.; Carpenter, J. E.; Weinhold, F. NBO Version3.1, TCI, University of Wisconsin, Madison, **1998.**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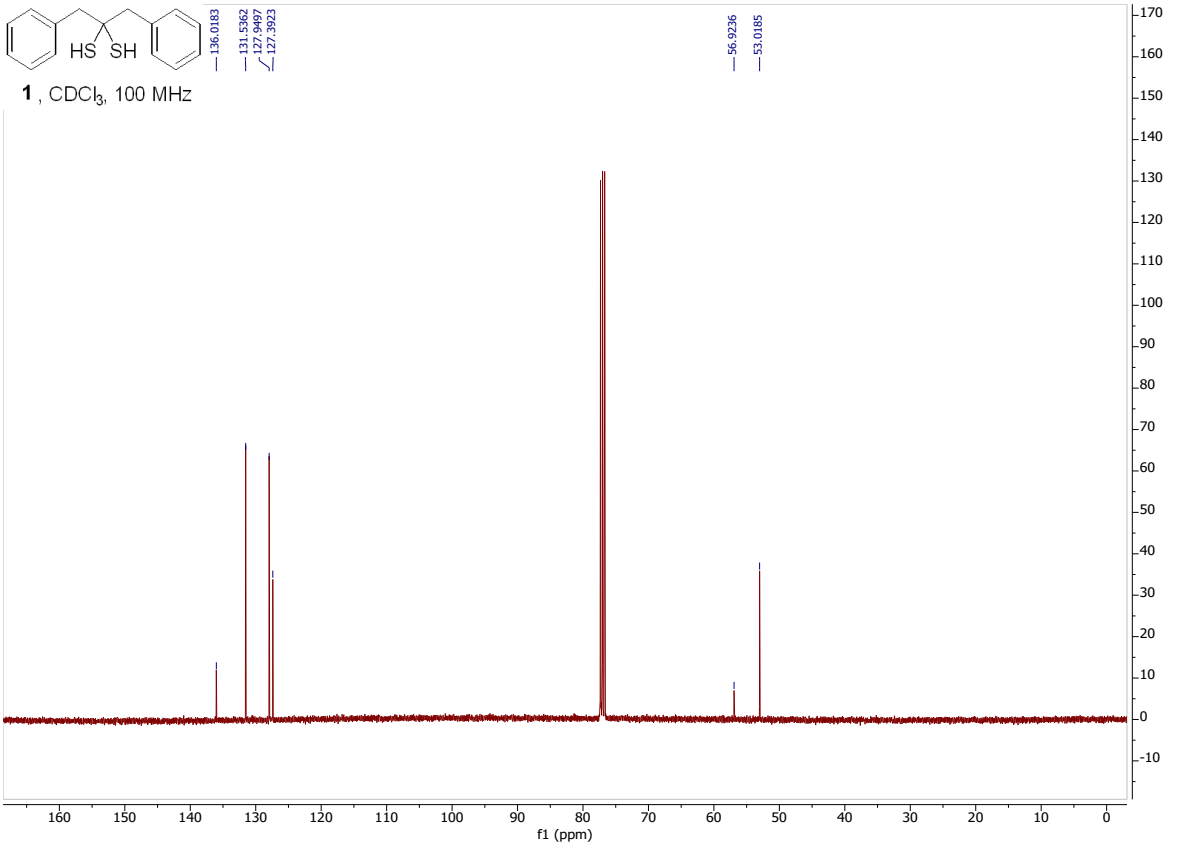
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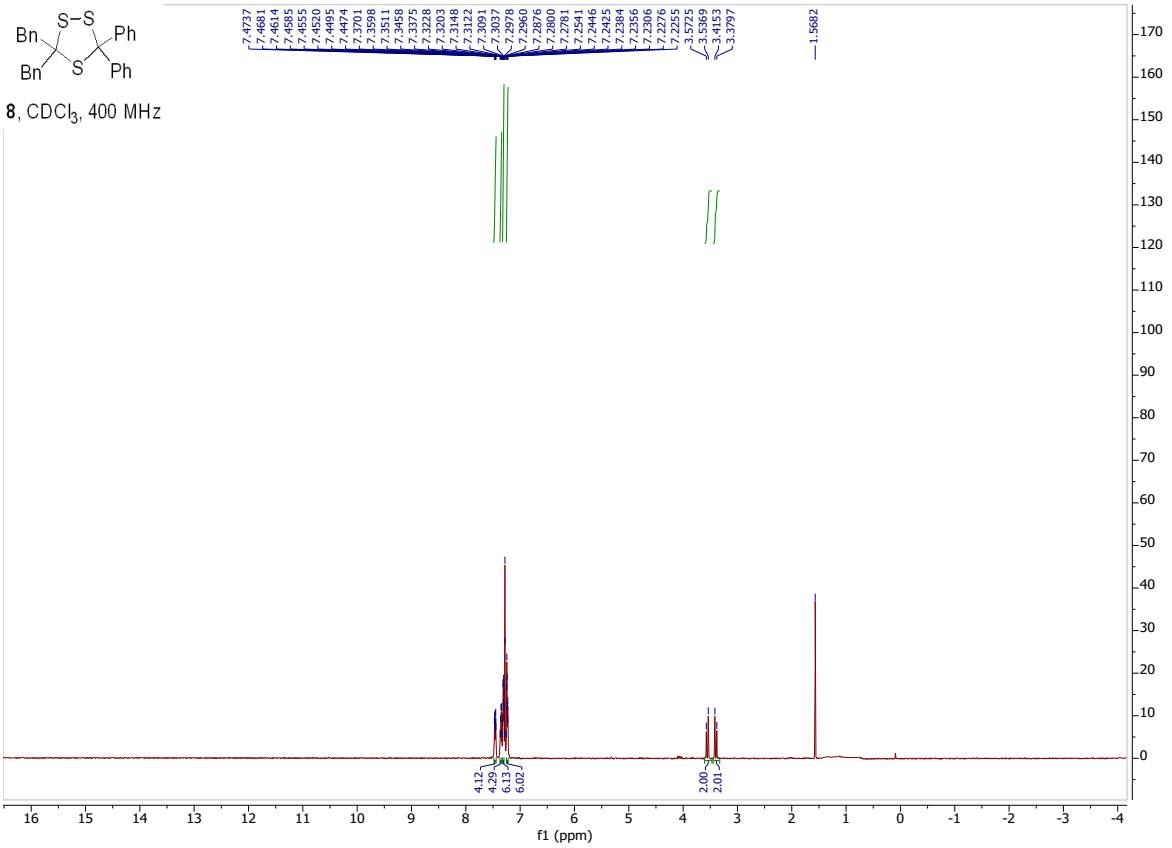
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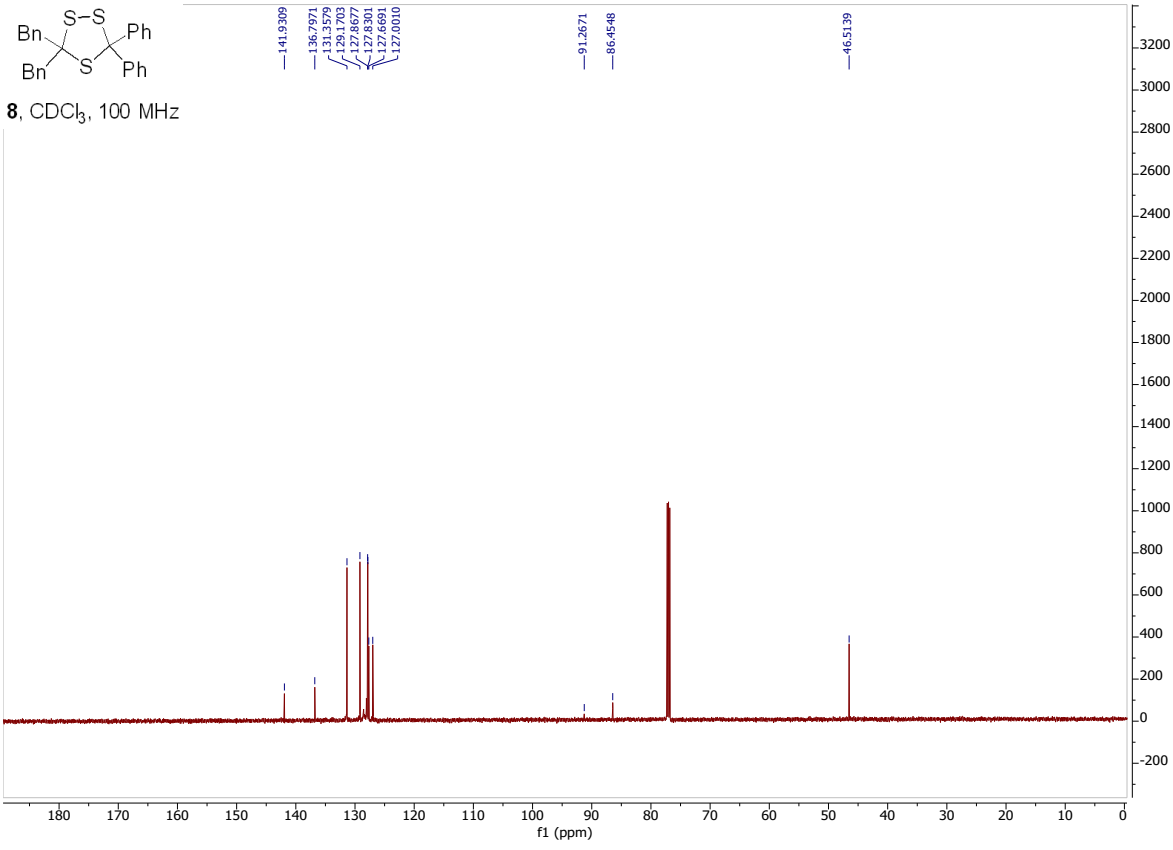
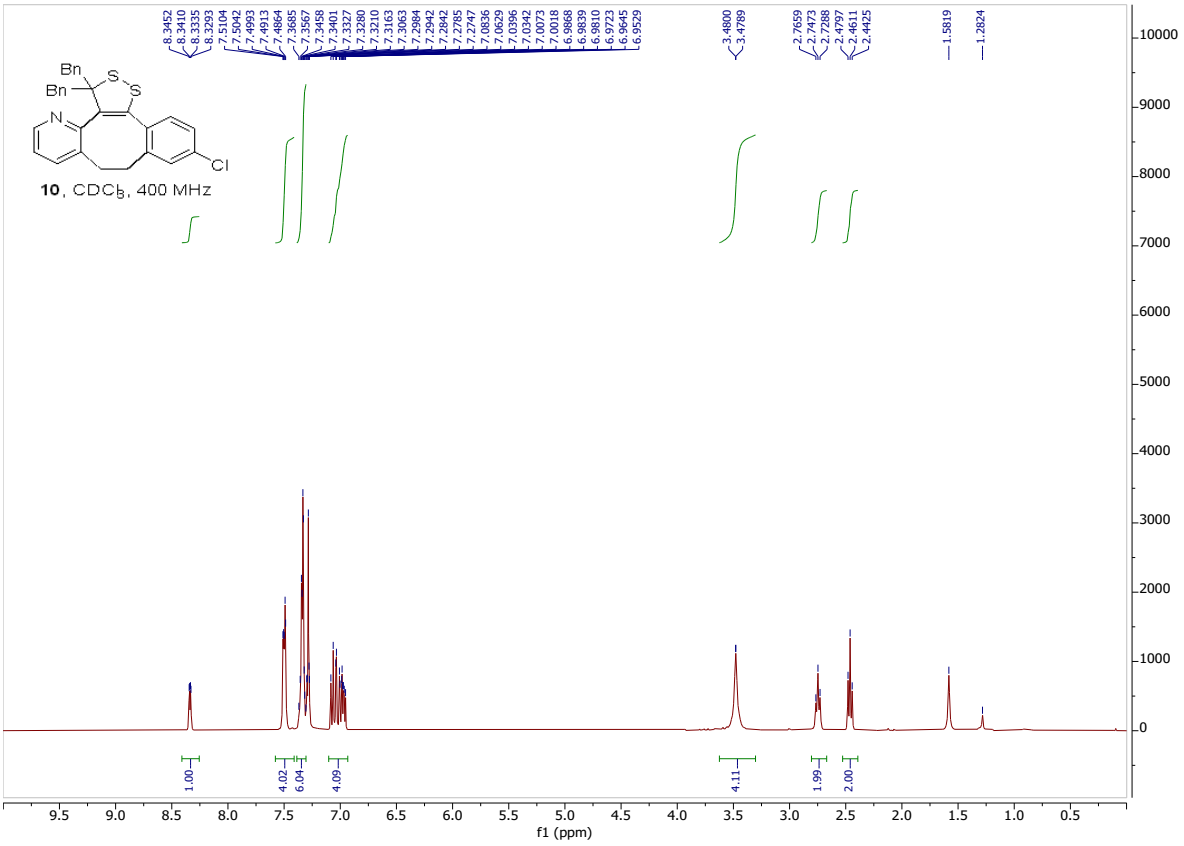
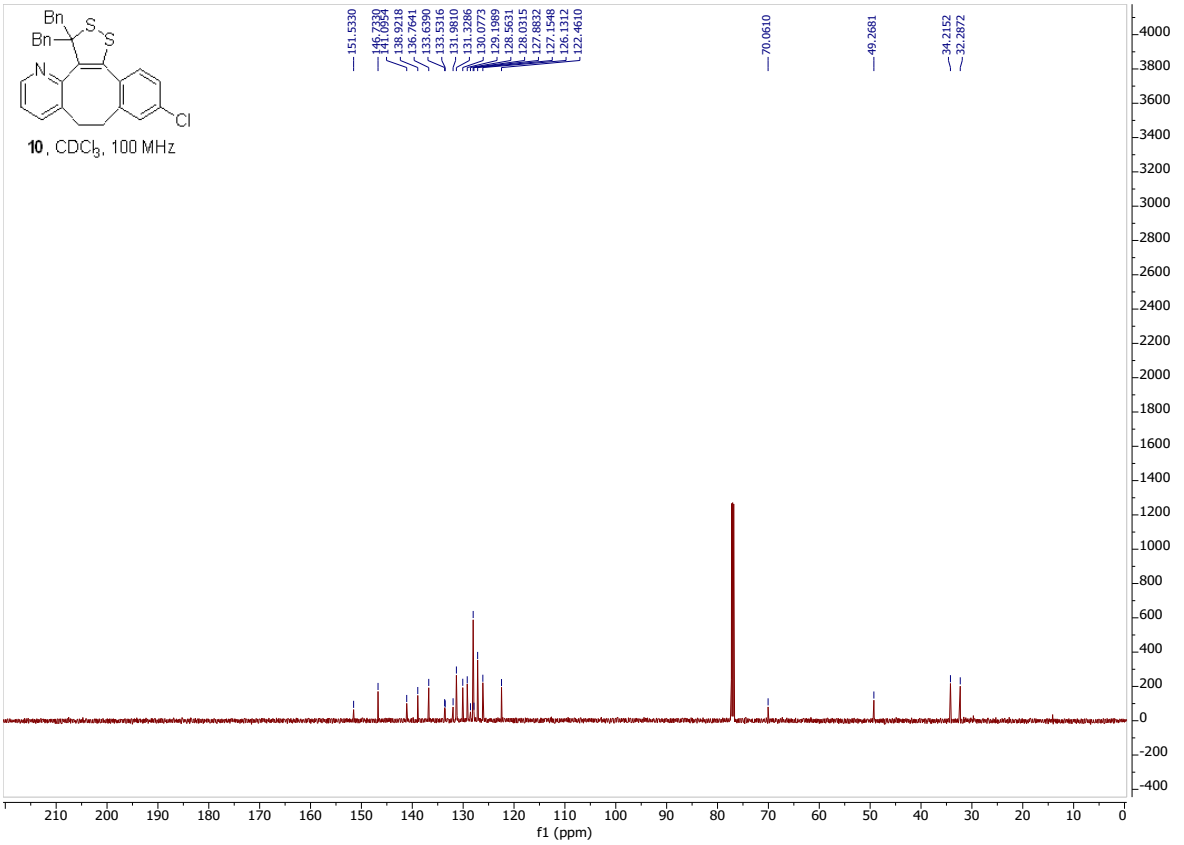

Bn
S–S
Ph
Bn
S
Ph
8, CDCl3, 100 MHz
141.9309
136.7971
131.3579
129.1703
127.8677
127.8301
127.6691
127.0010
91.2671
86.4548
46.5139
f1 (ppm)


10, CDCl3, 400 MHz
f1 (ppm)


10, CDCl3, 100 MHz
f1 (ppm)