A Prototype Reversible Polymersome-Stabilized H_2S Photoejector Operating Under Pseudophysiological Conditions

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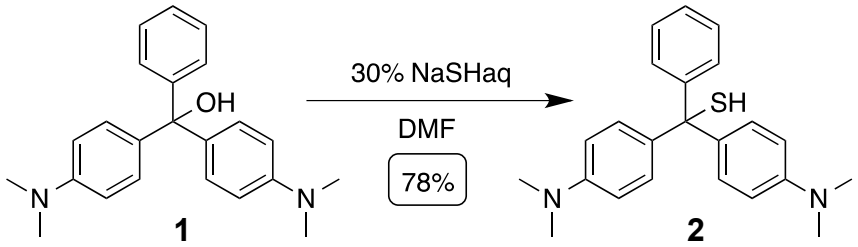
1. Materials and Methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz / 75 MHz). Proton chemical shifts (δ) are reported in ppm downfield from tetramethylsilane (TMS). Reagent grade tetrahydrofuran (THF) was distilled under argon over sodium benzophenone ketyl. CH_2Cl_2 was distilled over CaH_2 under argon. All other reagents were purchased from commercial suppliers and used without further purification. Flash column chromatography was carried out using silica gel (230-400 mesh). Thin layer chromatography (TLC) was carried out on plates coated with silica gel 40 F254 purchased from Aldrich. All reactions were conducted under dry oxygen free atmosphere using oven-dried glassware unless otherwise stated. **3** was prepared using literature procedures[S1] and $PBut_{22}$-*b*-PEO_{14} was obtained from Polymer Source. Dynamic Light Scattering (DLS) analyses were used to obtain the average size of vesicles after extrusion by using a Malvern ZetaSizer Nano ZS instrument with detection at 90°. Samples were analyzed at room temperature. Mass spectrometry was performed by the CESAMO analytical centre (University of Bordeaux, France) on a QStar Elite mass spectrometer (Applied Biosystems). The instrument is equipped with an ESI source and spectra were recorded in the positive mode. The electrospray needle was maintained at 5000 V and operated at room temperature. Samples were introduced by injection through a 20 μL sample loop into a 4500 μL/min flow of methanol from the LC pump. Electronic absorption spectra were measured on a Varian Cary 5000 UV-vis-NIR spectrophotometer. Steady-state emission spectra were recorded on a Horbiba Jobin-Yvon Fluorolog-3 spectrofluorometer equipped with a R928P PMT and were corrected.

Photo-dehydroxylation or Photo-dehydrosulfidation Quantum Yield. Photoreaction quantum yields were determined following excitation at 254, 313 and 365 nm, using the couple potassium ferrioxalate-phenanthroline as a chemical actinometer[S2] on an optical bench

equipped with a 150 W Hg-Xe lamp and a monochromator. Samples (320 μM) were stirred during the irradiation and the amount of converted material was determined spectrophotometrically at 10 s intervals following the appearance of the charge transfer absorption band of the ejector (λ_{max} = 618 nm).

2. Synthesis of 2



Scheme S1. Synthesis of **2**.

30% Sodium hydrosulfide (8 mmol) solution was added to a malachite green carbinol base (**1**) (1.1 g, 3.2 mmol) dissolved in DMF (12 mL). The mixture was stirred at 40°C for 2 h. The mixture was poured into 20 mL of ice water. The precipitates were collected by filtration and washed with sodium hydrosulfide solution (2%) and cold water. The crude products were dried and recrystallized from methanol to affording **2** as a white solid in 78% yield (730 mg). **^{1}H NMR** (300 MHz, $CDCl_3$, 25ºC): δ (ppm) = 7.29 (m, 5H), 7.11 (d, *J* = 8.7 Hz, 4H), 6.55 (d, *J* = 8.7 Hz, 4H), 2.94 (s, 12H), 2.63 (s, 1H). **^{13}C NMR** (75 MHz, $CDCl_3$, 25ºC): δ (ppm) = 149.5, 148.0, 135.7, 128.9, 127.9, 127.7, 126.7, 111.8, 81.6, 40.7. **HRMS** (EI) calcd for $C_{23}H_{27}N_2S$ *m*/*z* = 363.1889 $[M+H]^+$, found *m*/*z* = 363.1899 $[M+H]^+$; calcd for $C_{23}H_{25}N_2$ *m*/*z* = 329.2012 $[M-SH]^+$, found *m*/*z* = 329.2045 $[M-SH]^+$.

3. Polymersome vesicle preparation

$PBut_{22}$-*b*-PEO_{14} polymersomes were prepared by the film rehydration method. 10 mg $PBut_{22}$-*b*-PEO_{14} and 0.20 mg photoejector **1** or **2** were dissolved in 2 mL dichloromethane. The organic solvent was then removed by evaporation on a rotary evaporator. The residual thin polymer film was hydrated with 2 mL PBS under magnetic stirring for 3 hours to allow spontaneous formation of polydisperse and multilamellar vesicles. The resulting suspension was extruded through a 100 nm polycarbonate filter using a mini-extruder (Avanti Polar Lipids) and the obtained polymersomes were analyzed by DLS.

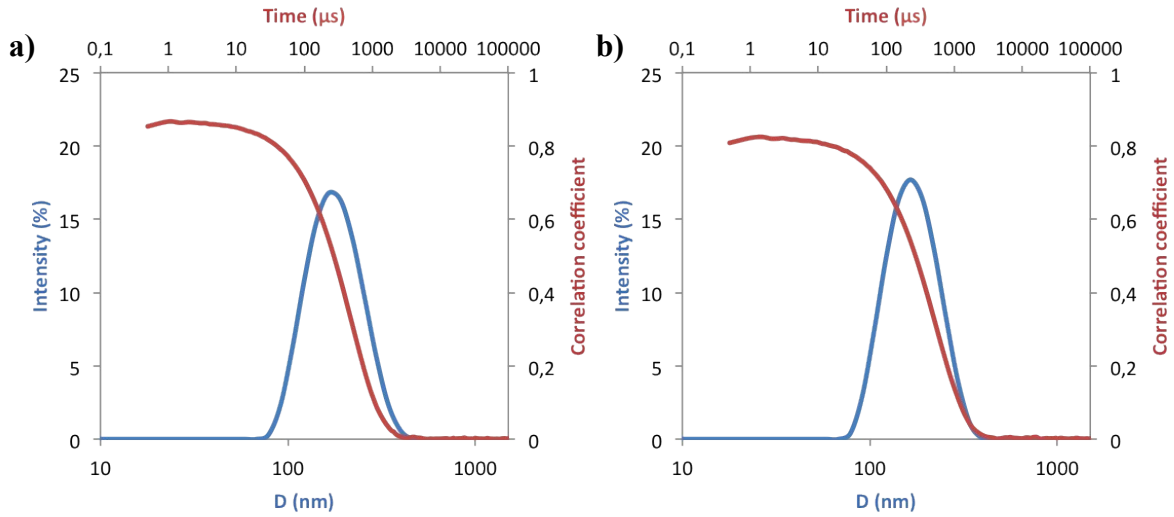


Figure S1. DLS intensity and correlation profiles after extrusion of $PBut_{22}$-*b*-PEO_{14} vesicles encapsulating a) **1** or b) **2**.

4. Electronic absorption and fluorescence spectra

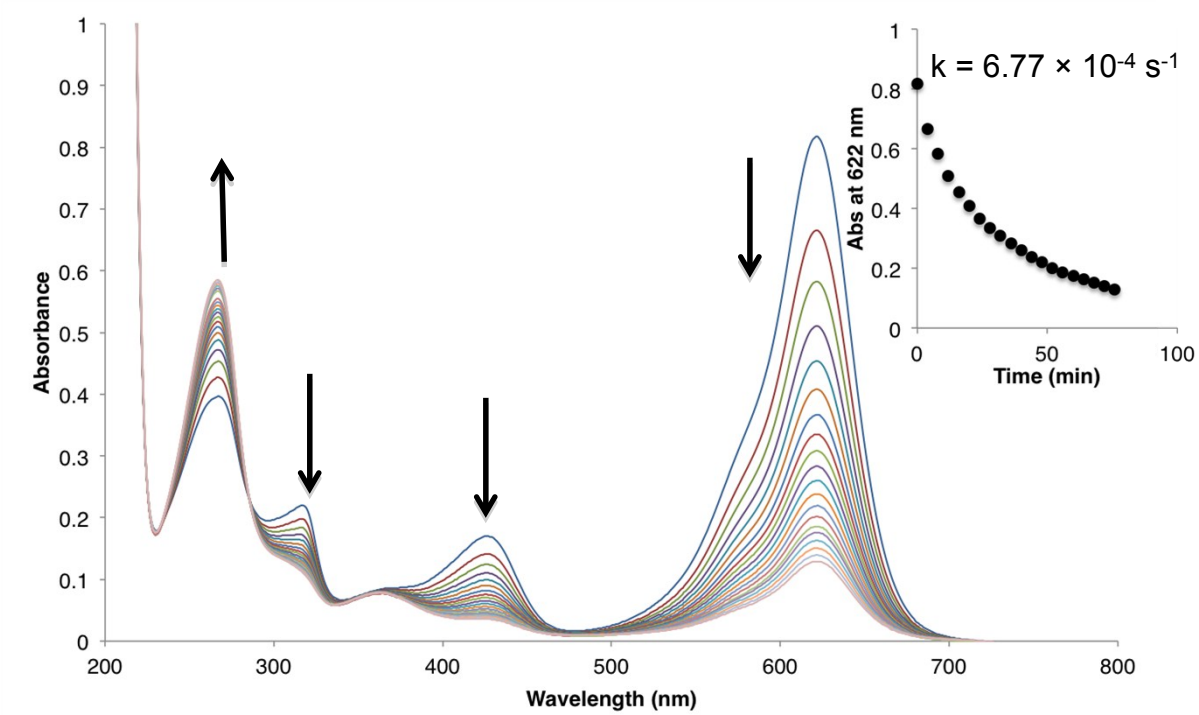


Figure S2. Spectral variations of a solution **2** (45 µM in toluene) following irradiation at 254 nm during 120 s, attributed to recombination. The insert shows the decrease in the absorption at 622 nm, according to first order kinetics with a rate constant of 6.77×10^{-4} s^{-1}.

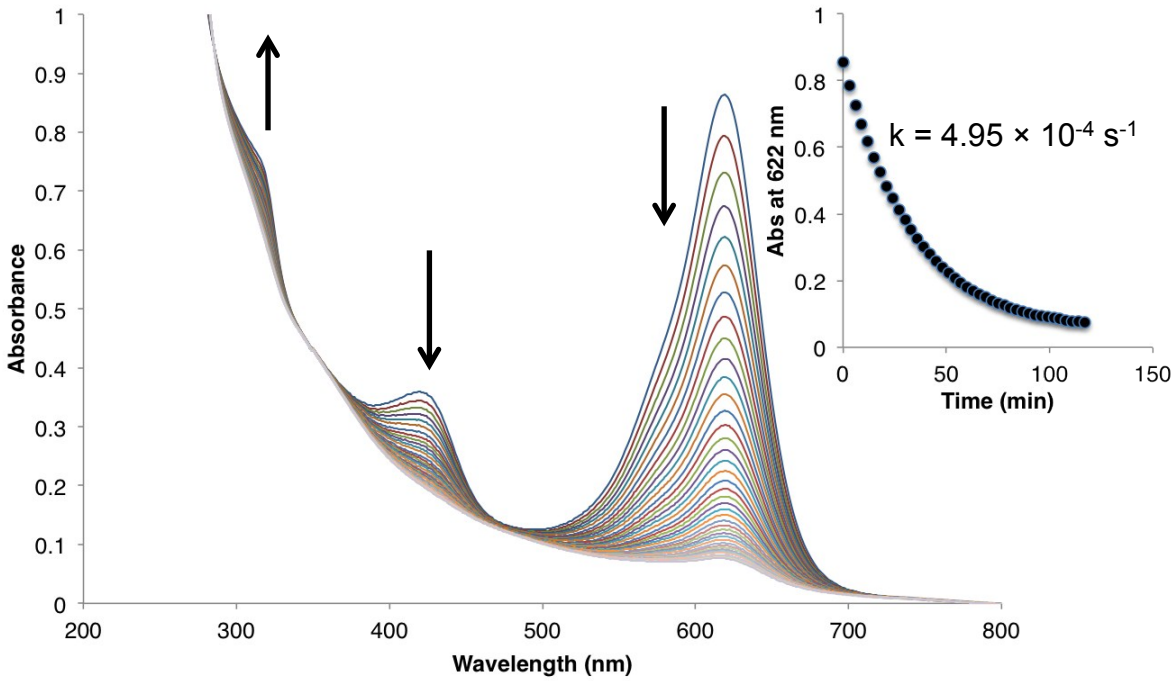


Figure S3. Spectral variations of a solution of **2** (48.3 µM in $PBut_{22}$-*b*-PEO_{14} polymersomes) following irradiation at 254 nm during 90 s, attributed to recombination. The insert shows the decrease in the absorption at 622 nm, according to first order kinetics with a rate constant of 4.95×10^{-4} s^{-1}.

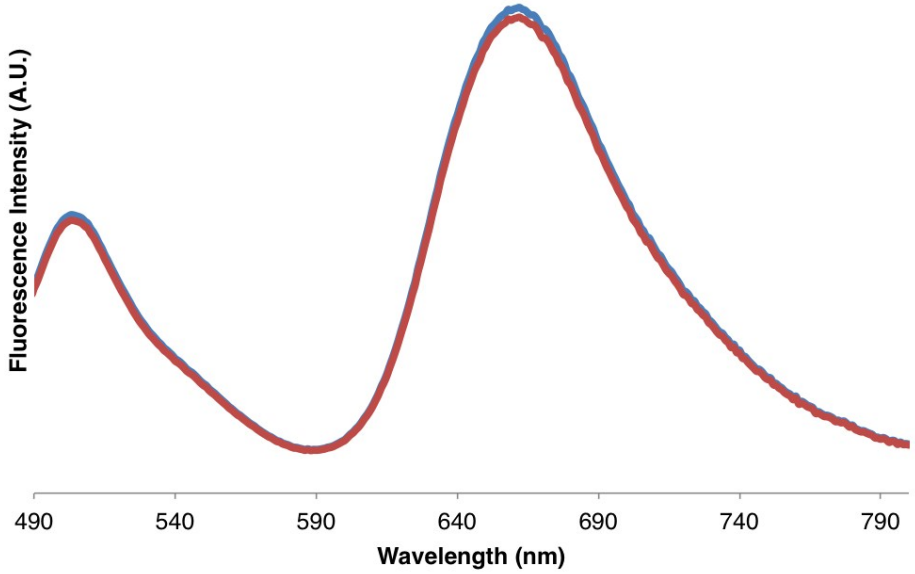


Figure S4 Fluorescence spectra (λ_{exc} = 475 nm) of fluorescent probe **3** (4.2 µM) in PBS (20 mM, pH 7.4, 2% DMF, *v*/*v*) (blue) and a mixture of **2** (48.3 µM in $PBut_{22}$-*b*-PEO_{14} polymersomes) and fluorescent probe **3** (4.2 µM) in PBS (20 mM, pH 7.4, 2% DMF, *v*/*v*) (red).

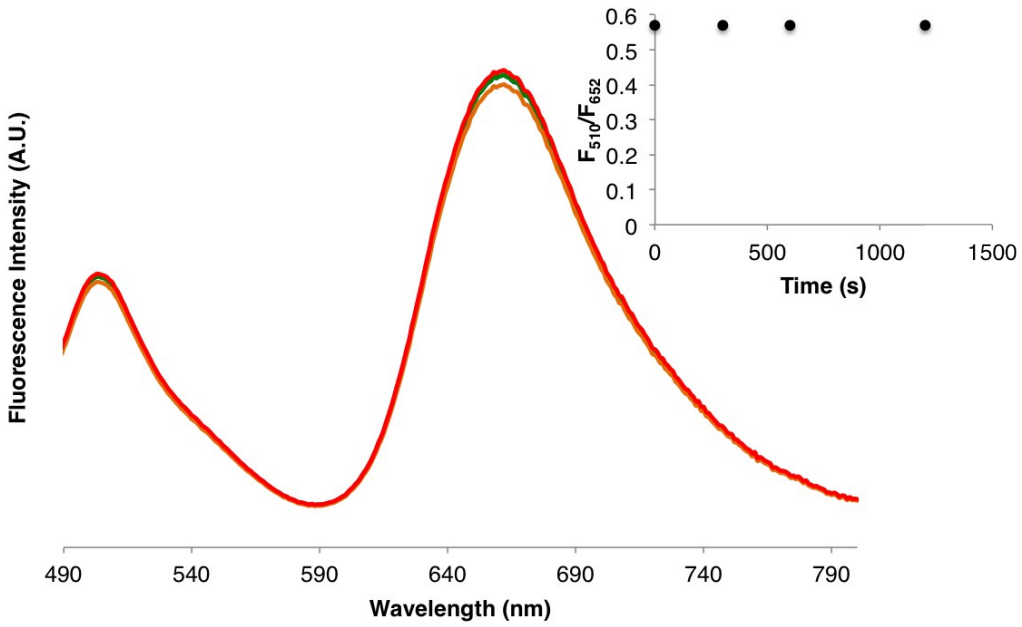


Figure S5. Fluorescence spectra (λ_{exc} = 475 nm) of fluorescent probe **3** (4.2 μM) in PBS (20 mM, pH 7.4, 2% DMF, *v*/*v*) obtained upon irradiation at 254 nm from 0 to 1200 s.

5. Response time of Probe after irradiation of photoejector

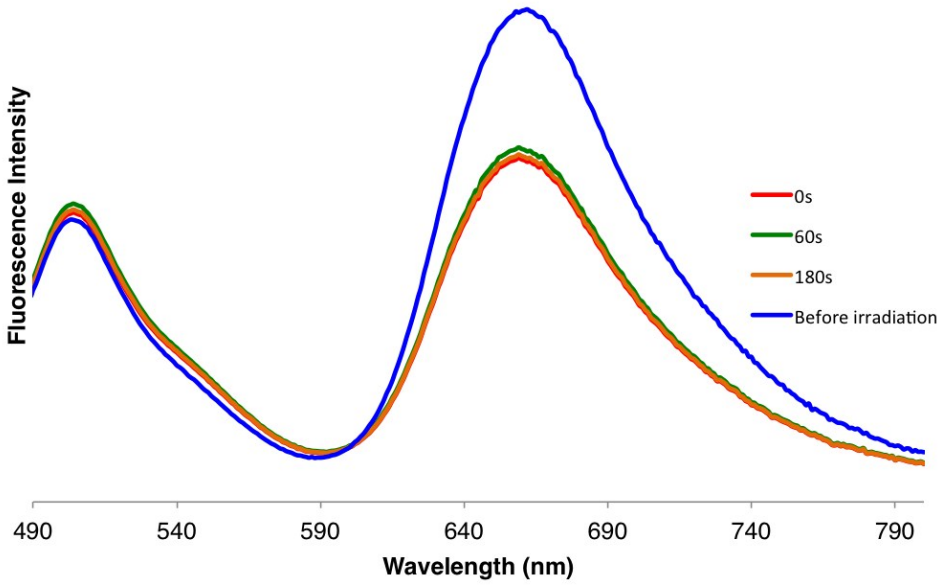


Figure S6. Fluorescence spectra (λ_{exc} = 475 nm) of **2** (48.3 μM in $PBut_{22}$-*b*-PEO_{14} polymersomes) and fluorescent probe **3** (4.2 μM) in PBS (20 mM, pH 7.4, 2% DMF, *v*/*v*) determined at 60s and 180s after 10s of irradiation at 254 nm.

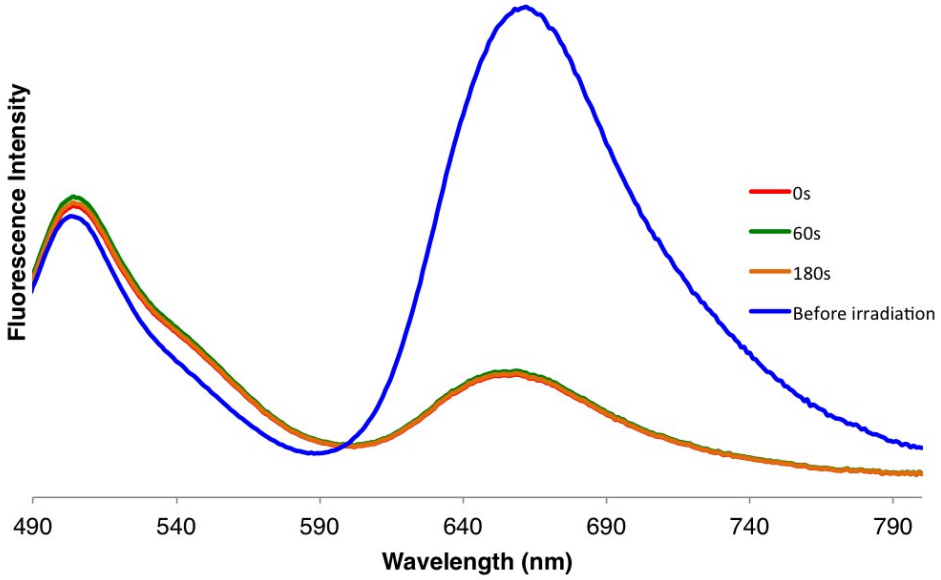


Figure S7. Fluorescence spectra (λ_{exc} = 475 nm) of **2** (48.3 μM in $PBut_{22}$-*b*-PEO_{14} polymersomes) and fluorescent probe **3** (4.2 μM) in PBS (20 mM, pH 7.4, 2% DMF, *v*/*v*) determined at 60s and 180s after 40s of irradiation at 254 nm.

Supporting Information References

[S1] Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L. Qiu, J. Cen and Z. Guo, *Angew. Chem. Int. Ed.* 2013, **52**, 1688-1691.

[S2] M. Montalti, A. Credi, L. Prodi and M. T. Gandolfi, *Handbook of Photochemistry*, Taylor et Francis, pp 601-604.

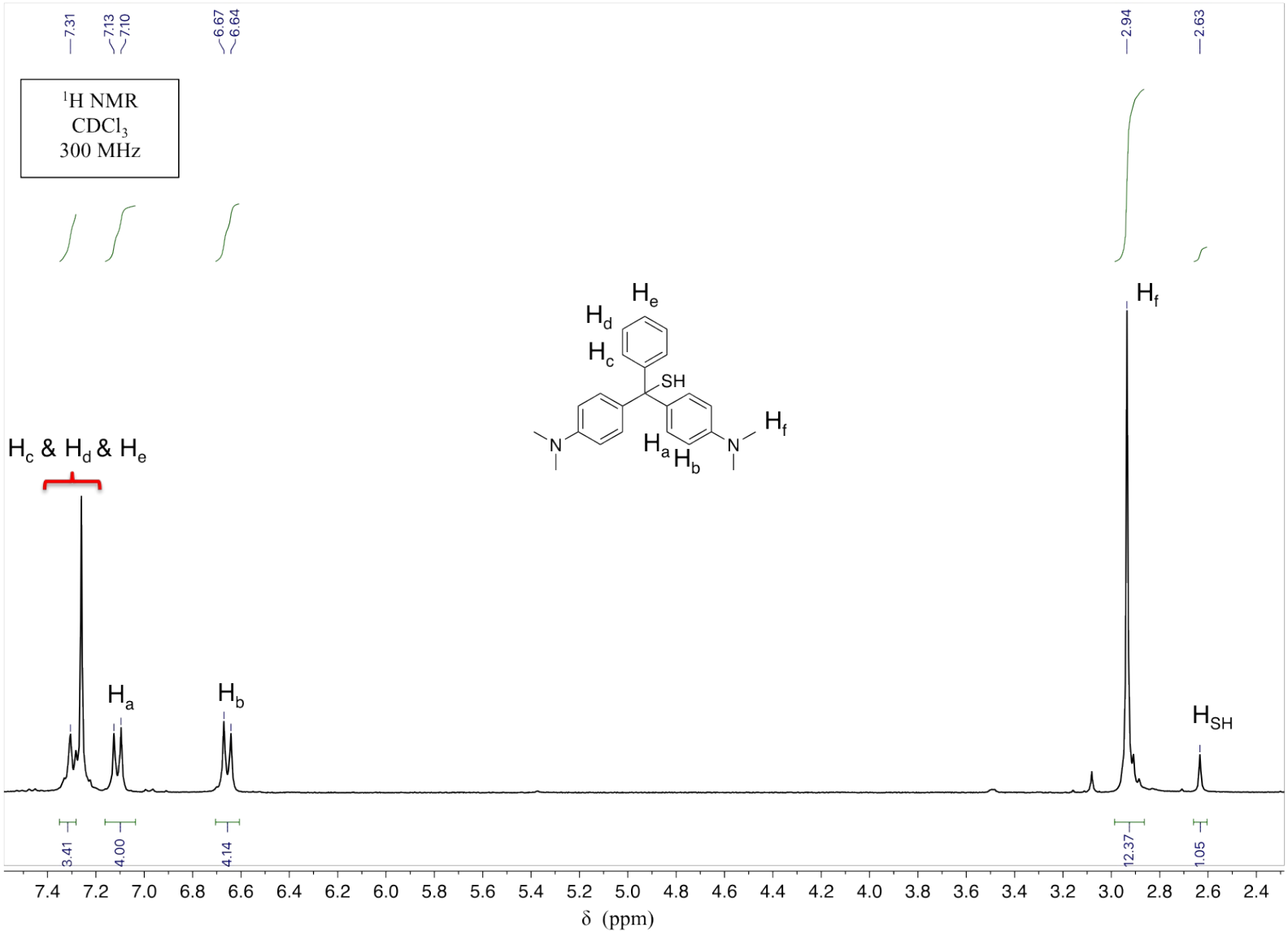


Figure S8. ^{1}H NMR spectrum of **2** in $CDCl_3$ at 298 K on a 300 MHz spectrometer.

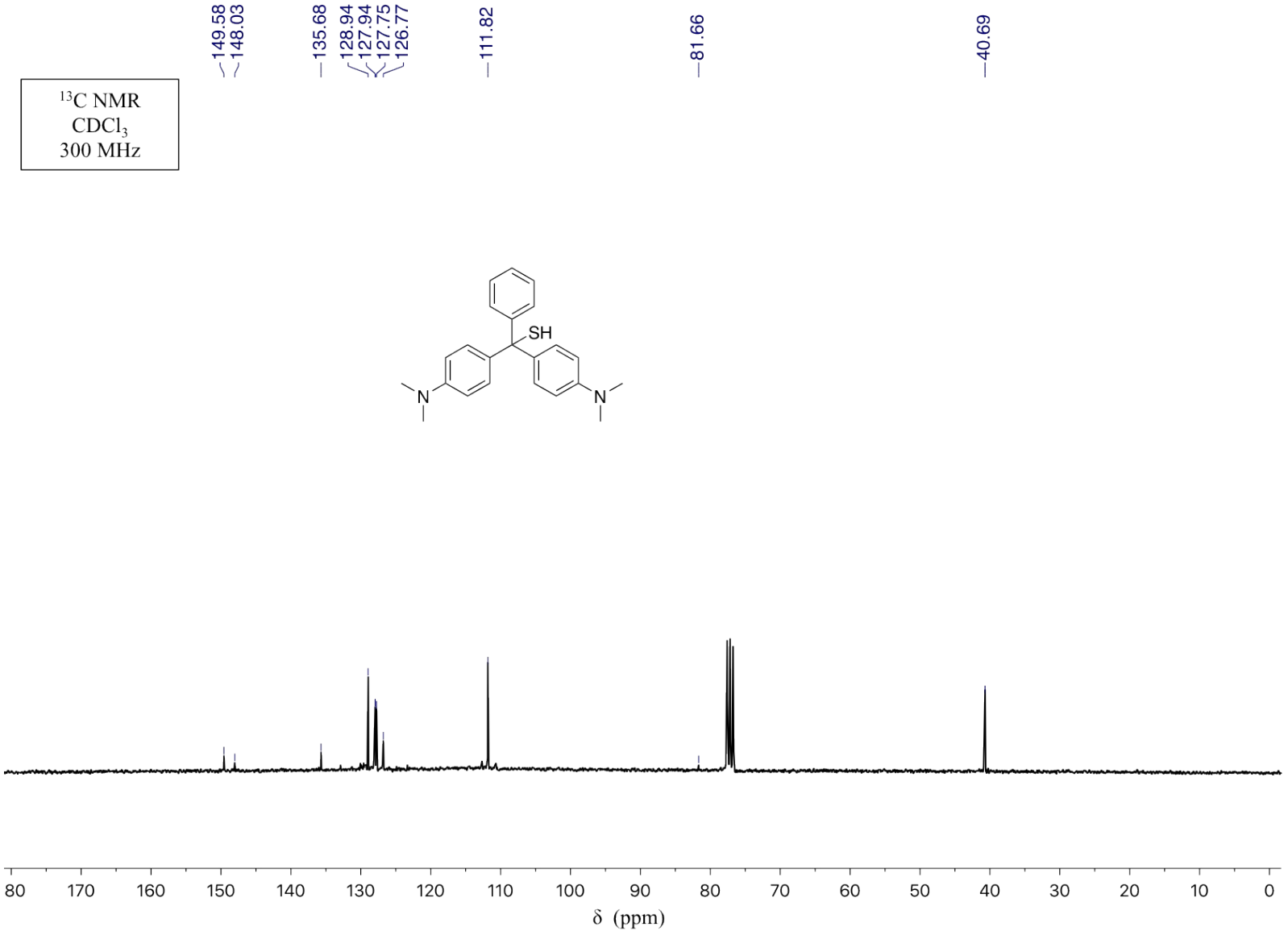


Figure S9. ^{13}C NMR spectrum of **2** in $CDCl_3$ at 298 K on a 300 MHz spectrometer.