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Supporting Information

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Multi-stimuli mediated release of chloride ion carrier from sulfonium-linked water-soluble procarrier

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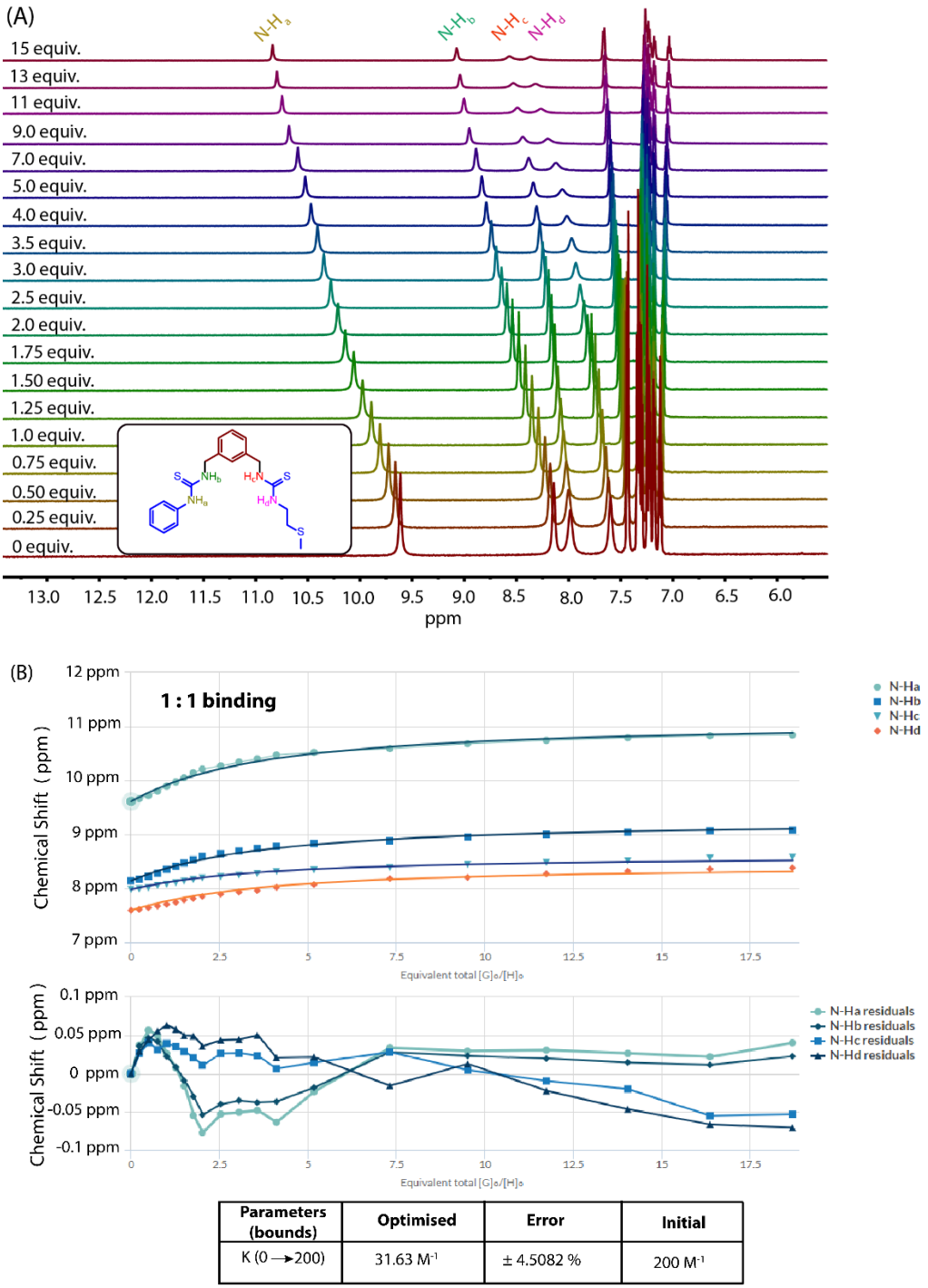
1. General information.

All the chemicals were purchased from commercial sources like Sigma Aldrich and Alfa Aesar and directly used without further purification. Thin-layer chromatography (TLC) on silica gel 60 F254 (0.25 mm) was used to monitor completion of the reactions, and column chromatography was performed using the silica gel of 120-200 mesh (Merck). The ^{1}H NMR and ^{13}C NMR were recorded at 600 MHz and 151 MHz, respectively, by Bruker spectrometer. The chemical shifts were reported in parts per million (**δ**) using DMSO-d_6, $CDCl_3$, as the internal solvent. The coupling constant (*J*) values were reported in Hz, and the abbreviation was stated as follows: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiple). High-resolution mass spectra (HRMS) were recorded at Agilent Q-TOF mass spectrometer with a Z-spray source using built-in software for the analysis of the documented mass data. Egg yolk phosphatidylcholine (EYPC) and cholesterol used for the biophysical assays were purchased from Sigma Aldrich. HEPES buffer, 8-hydroxypyrene-1, 3, 6-trisulfonic acid (HPTS), calcein, Triton X-100, and inorganic salts were also obtained from Sigma Aldrich. Ultrapure water (Milli-Q system, Millipore, Billerica, MA) was used for the preparation of all the buffers, and stock solutions of all the compounds were prepared in gas chromatographic grade DMSO which also purchased from Sigma.

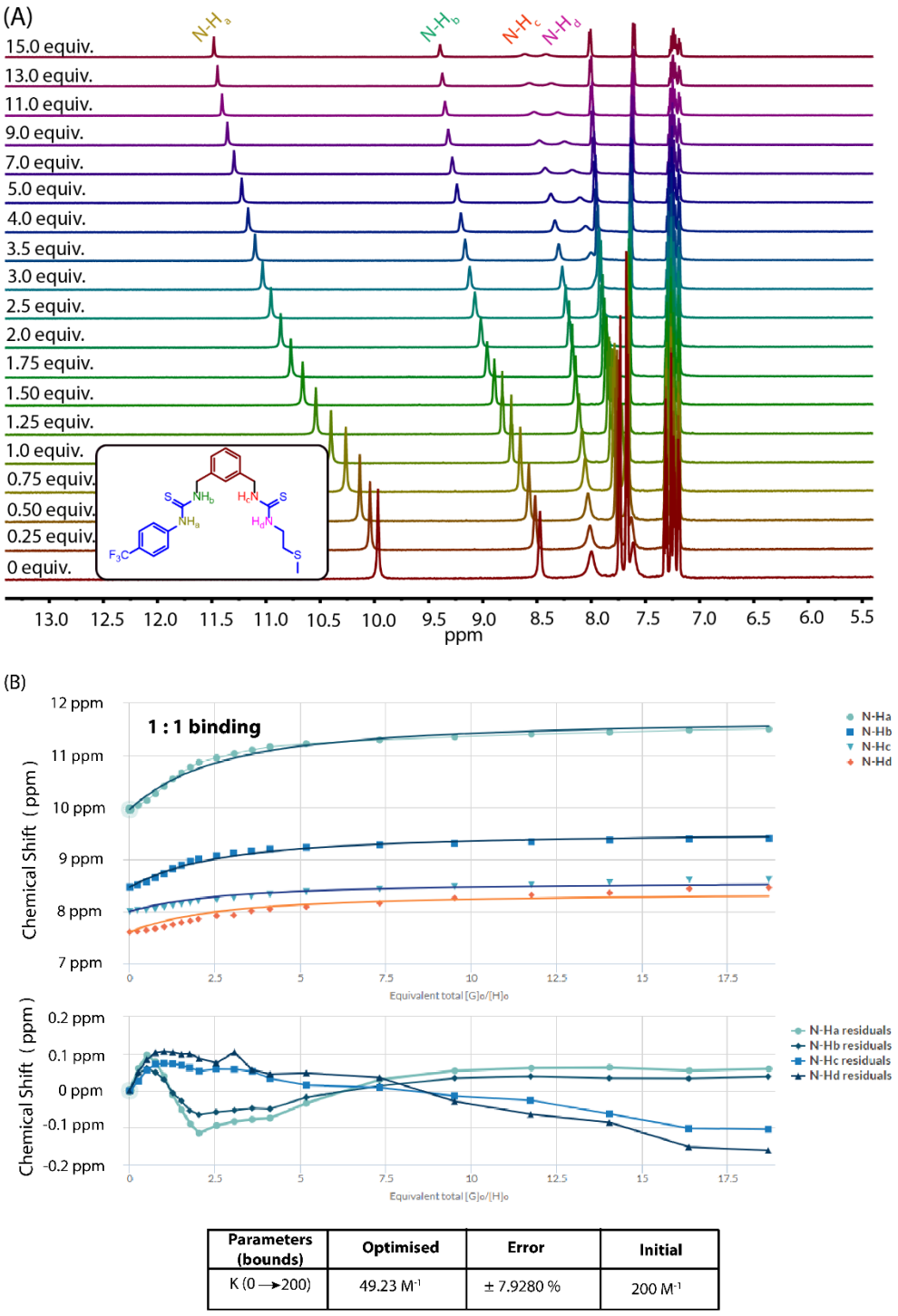
2. Anion binding analysis by ^{1}H-NMR titration.

The ^{1}H NMR titration was performed for compounds **1a**, **1b**, **1c,** and **1d** in DMSO-d_6. The stock solutions of the compound (10 mM) and tetrabutylammonium chloride (TBACl; 15 M) were prepared in DMSO d_6. The TBACl was used as the source of Cl^- ion. The ^{1}H NMR titrations of compounds in DMSO-d_6 were performed by the subsequent addition of TBACl (0-35 equiv.). The changes in chemical shift (Δδ) values of the N-H protons of the compounds were analyzed. MestReNova software was used for the stacking of all the titration spectra. The BindFit v0.5 software fitted the changes in a chemical shift against the concentration of chloride ion. The association constant (K_a) values were calculated using the BindFit v0.5 software (1:1 binding model).



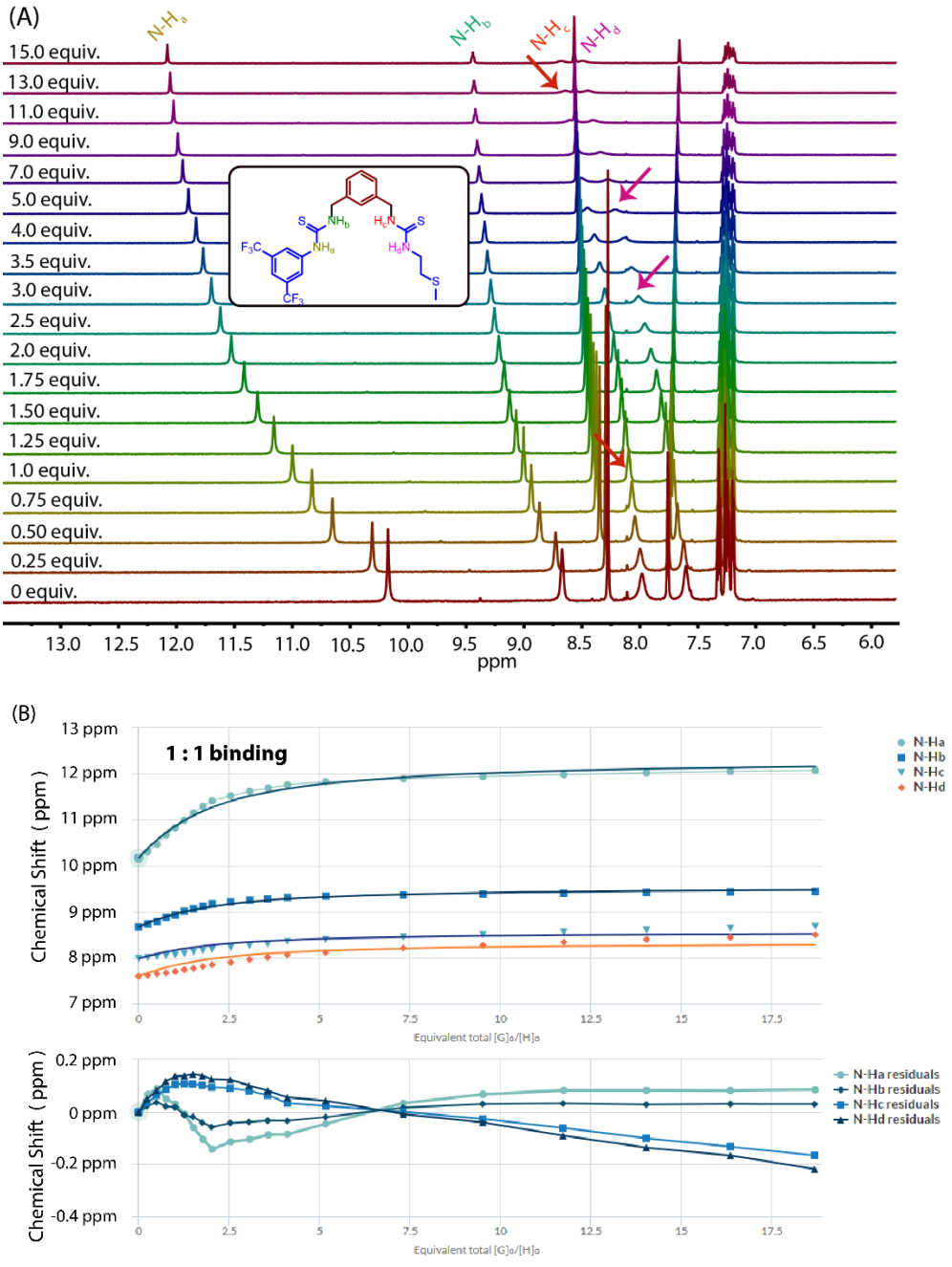
Parameters (bounds)	Optimised	Error	Initial
K (0 ⟶200)	31.63 M^{-1}	± 4.5082 %	200 M^{-1}

Fig. S1. ^{1}H-NMR titration spectra for compound **1a** (10 mM) with the sequential addition of TBACl in DMSO-d_6 solvent. The amounts of added TBACl are shown on the spectra (**A**). The plot of chemical shift (δ) of N-H_a and N-H_b protons *vs.* equivalent total ($[G]_0/[H]_0$) added, fitted to 1:1 binding model of BindFit v0.5 program (**B**). H = host = **1a** and G = guest = TBACl.



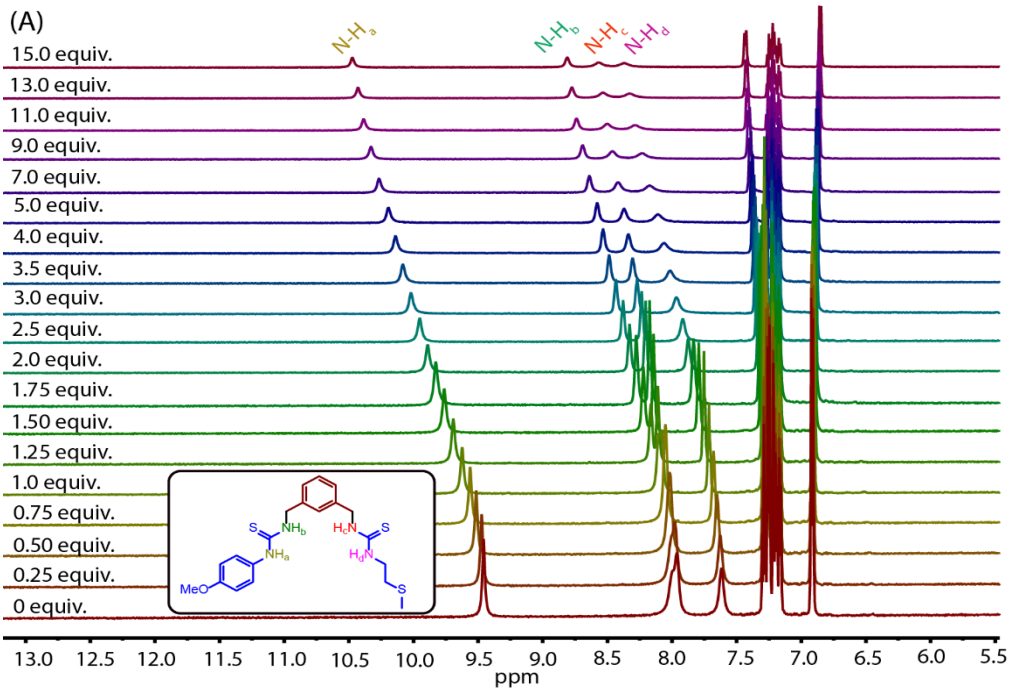
Parameters (bounds)	Optimised	Error	Initial
K (0 → 200)	49.23 M^{-1}	± 7.9280 %	200 M^{-1}

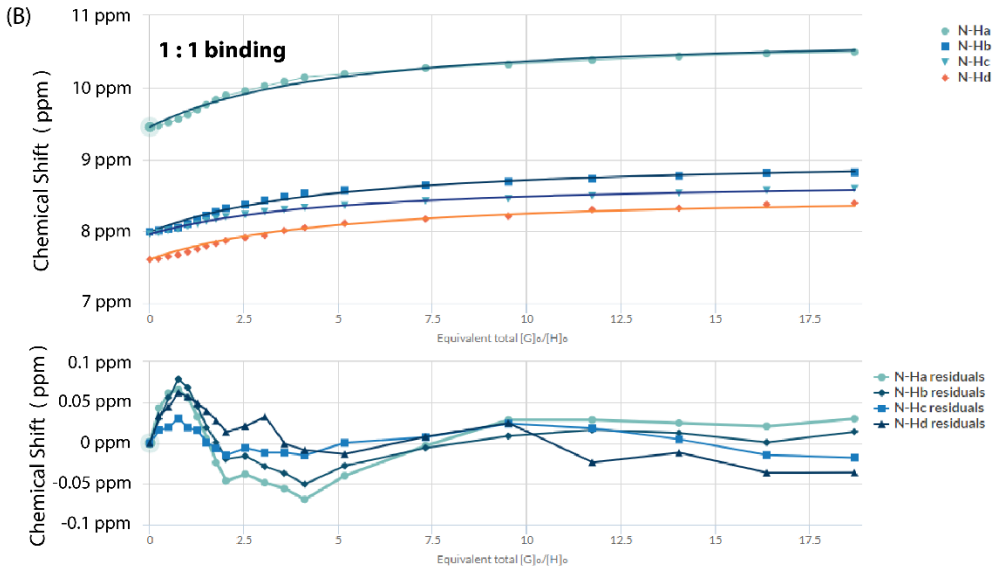
Fig. S2. ^{1}H-NMR titration spectra for compound **1b** (10 mM) with the sequential addition of TBACl in DMSO-d_6 solvent. The amounts of added TBACl are shown on the spectra (**A**). The plot of chemical shift (δ) of N-H_a and N-H_b protons *vs.* equivalent total ($[G]_0/[H]_0$) added, fitted to 1:1 binding model of BindFit v0.5 program (**B**). H = host = **1b** and G = guest = TBACl.



Parameters (bounds)	Optimised	Error	Initial
K (0 ⟶ 200)	68.57 M^{-1}	± 9.6246 %	200 M^{-1}

Fig. S3. ^{1}H-NMR titration spectra for compound **1c** (10 mM) with the sequential addition of TBACl in DMSO-d_6 solvent. The amounts of added TBACl are shown on the spectra (**A**). The plot of chemical shift (δ) of N-H_a and N-H_b protons *vs.* equivalent total ($[G]_0/[H]_0$) added, fitted to 1:1 binding model of BindFit v0.5 program (**B**). H = host = **1c** and G = guest = TBACl.





Parameters (bounds)	Optimised	Error	Initial
K (0 → 100)	24.31 M^{-1}	± 4.0612 %	100 M^{-1}

Fig. S4. ^{1}H-NMR (600MHz) titration spectra for compound **1d** (10 mM) with the sequential addition of TBACl in DMSO-d_6 solvent. The amounts of added TBACl are shown on the spectra (**A**). The plot of chemical shift (δ) of N-H_a and N-H_b protons *vs.* equivalent total ($[G]_0/[H]_0$) added, fitted to 1:1 binding model of BindFit v0.5 program (**B**). H = host = **1d** and G = guest = TBACl.

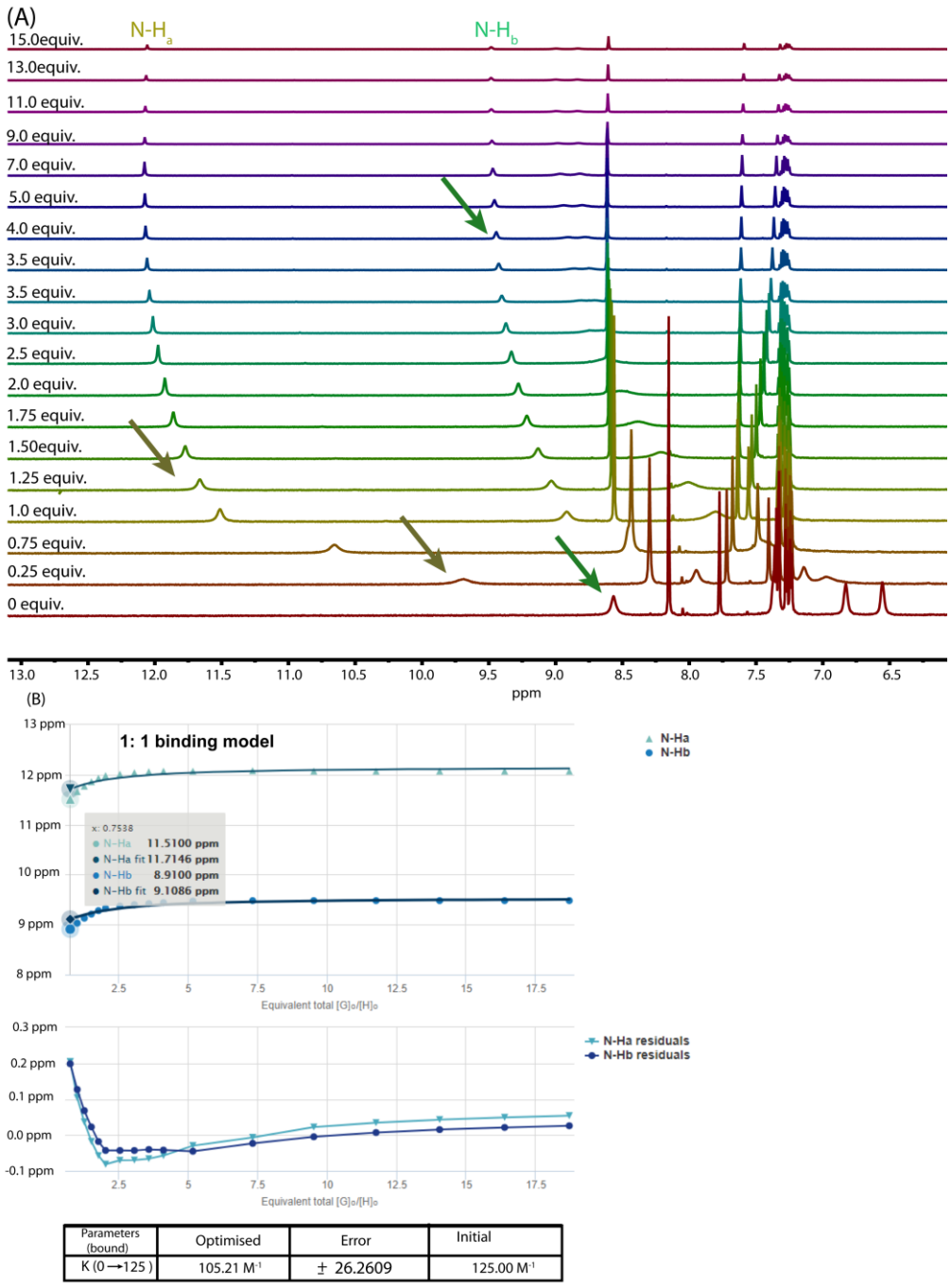


Fig. S5. ^{1}H-NMR titration spectra for compound **1c** (10 mM) with the sequential addition of TBACl in CD_3CN solvent. The amounts of added TBACl are shown on the spectra (**A**). The plot of chemical shift (δ) of N-H_a and N-H_b protons *vs.* equivalent total ($[G]_0/[H]_0$) added, fitted to 1:1 binding model of BindFit v0.5 program (**B**). H = host = **1c** and G = guest = TBACl.

Table S1. Binding properties of the compounds.

Compound	Binding constant (M^{-1})			Average(M^{-1})	Standard deviation(M^{-1})
	Set 1	Set2	Set 3		
1a	31.63	32.35	32.23	32.07	0.31
1b	49.23	49.88	49.43	49.51	0.27
1c	68.57	69.10	69.56	69.08	0.40
1d	24.31	25.10	24.32	24.58	0.37

Table S2. Binding properties of the compounds.

Compound	Aqueous solubility (mg/mL)
1a	Insoluble
1b	Insoluble
1c	Insoluble
1d	Insoluble
2	2.8

3. Ion transporting activity studies.

3.1. ***Preparation of EYPC-LUVs⊃HPTS*** **—** HPTS encapsulated large unilamellar vesicles (LUVs) were prepared by mixing 50 µL of EYPC (100 mg/mL in $CHCl_3$), and 80 µL of cholesterol (25 mg/mL in $CHCl_3$) were taken in a clean and dry glass vial in the molar ratio 6:4 (EYPC: cholesterol). The solution was then dried under vacuum for minimum 5 hours to form a thin monolayer film after which the thin film was rehydrated using 800 µL buffer (20 mM HEPES, pH 7.2 containing 100 mM NaCl and 1 mM HPTS) and kept for 1 hour at room temperature with occasional vortexing (6-7 times). It was then subjected to 17-19 freeze-thaw cycles and vortexed for the next 15 min. Finally, the suspension was extruded using a mini extruder (a polycarbonate membrane from Avanti Polar Lipids) having 200 nM as the pore size for 19-21 times (must be an odd number). Eventually, to ensure the removal of all the free/unencapsulated HPTS dye from the lipid suspension, the gel filtration (Sephadex G-50) column chromatography was performed with 20 mM HEPES buffer, pH 7.2 containing 100 mM NaCl. The final EYPC-LUV⊃HPTS concentration obtained was 25 mM (assuming 100 % lipid regeneration).

3.2. ***Ion transport activity across EYPC/CHOL-LUV⊃HPTS*** **—** The HPTS fluorescence assay was done by adding 2890 µL of buffer solution (20 mM HEPES buffer, pH 7.2, containing 100 mM NaCl), 50 µL of EYPC/CHOL-LUV⊃HPTS and 50 µL of 0.5 M NaOH in a clean and dry 3 mL fluorescence cuvette placed in the fluorescence spectrophotometer (Fluoromax-4 spectrofluorometer) at room temperature under the slow stirring condition for around 3 minutes. During this time, a pH gradient of ~ 0.6 between the extra- and intravesicular systems was generated. The HPTS fluorescence intensity was monitored (at t = 0 s) at λ_{em} = 510 nm (λ_{ex} = 450 nm). The kinetics was initiated at t = 50 s by adding 10 µL of the respective compound from their DMSO stock solution. The vesicles were lysed at t = 450 s by adding 20 µL 20% Triton X-100 solution, and the fluorescence measurement was continued for an additional 50 s (t = 500 s).

3.3. ***Quantitative measurement of transport activity from HPTS assay*** **—** The fluorescence emission intensities of the HPTS dye was normalized, and the intensities were appearing at t = 0 and t = 500 s was taken as 0 and 100 units, respectively. The normalized fluorescent intensities (FI) at t = 450 s (before the addition of Triton X-100 solution) were considered to measure the transport activity of the compounds.

$$i.e.\ Transport\ activity,\ \ T_{HPTS} = \frac{F_t - F_0}{(F_\infty - F_0)} \times 100\ \%$$ Eq.-S1

Where, F_t = fluorescence intensity at t = 450 s (prior to the addition of Triton X-100 solution), F_0 = fluorescence intensity immediately before the addition of the NaOH (t = 0 s) and F_∞ =

fluorescence intensity after addition of Triton X-100 solution (i.e. at saturation after complete leakage at t = 500 s).

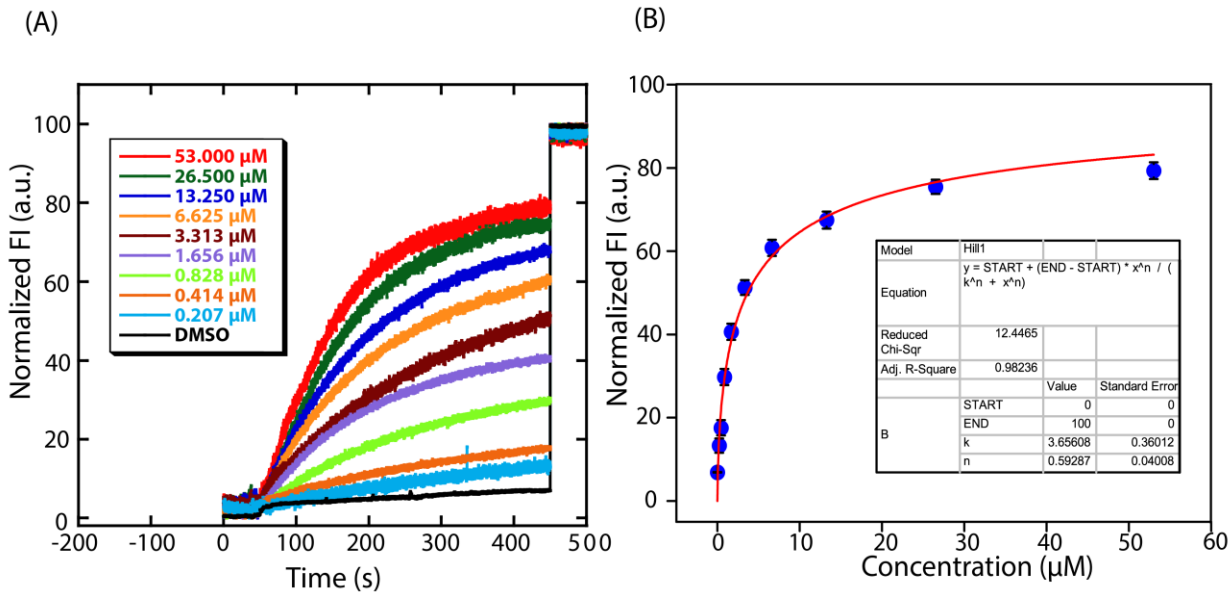


Fig. S6. Concentration-dependent transmembrane transport of Cl^- ion in the presence of compound **1a** across the EYPC/CHOL-LUV⊃HPTS. The ion transport activity was measured by HPTS fluorescence assay using a pH gradient (pH_{in} = 7.2, pH_{out} = 7.8; A). The EC_{50} value was calculated using the modified Hill equation (B).

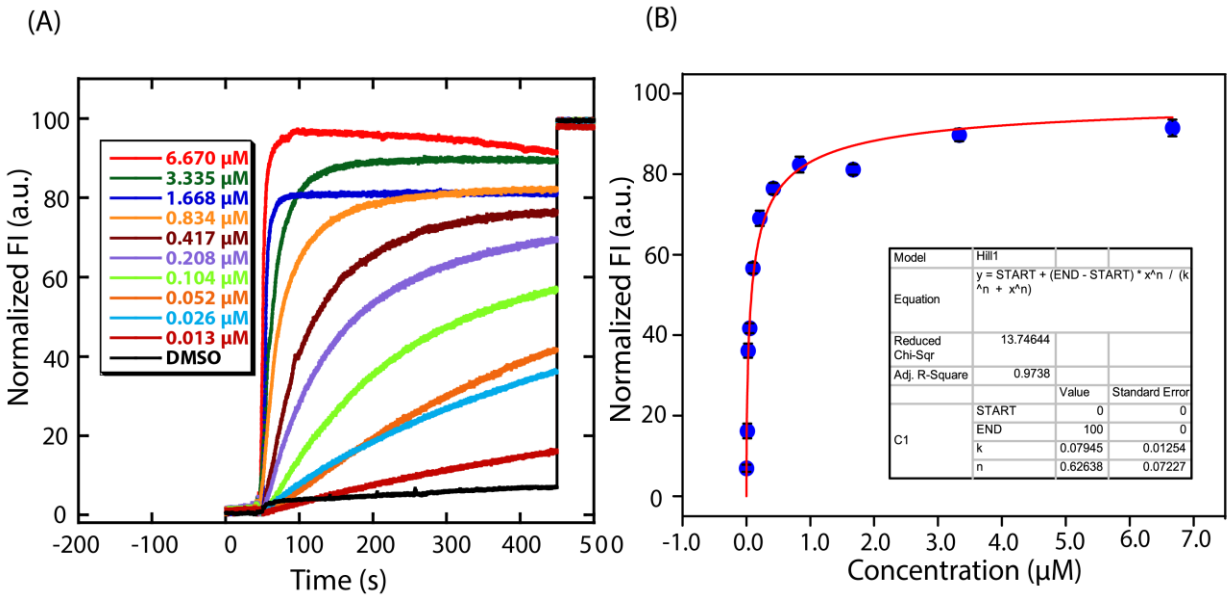


Fig. S7. Concentration-dependent transmembrane transport of Cl^- ion in the presence of compound **1b** across the EYPC/CHOL-LUV⊃HPTS. The ion transport activity was measured by HPTS fluorescence assay using a pH gradient (pH_{in} = 7.2, pH_{out} = 7.8; A). The EC_{50} value was calculated using the modified Hill equation (B).

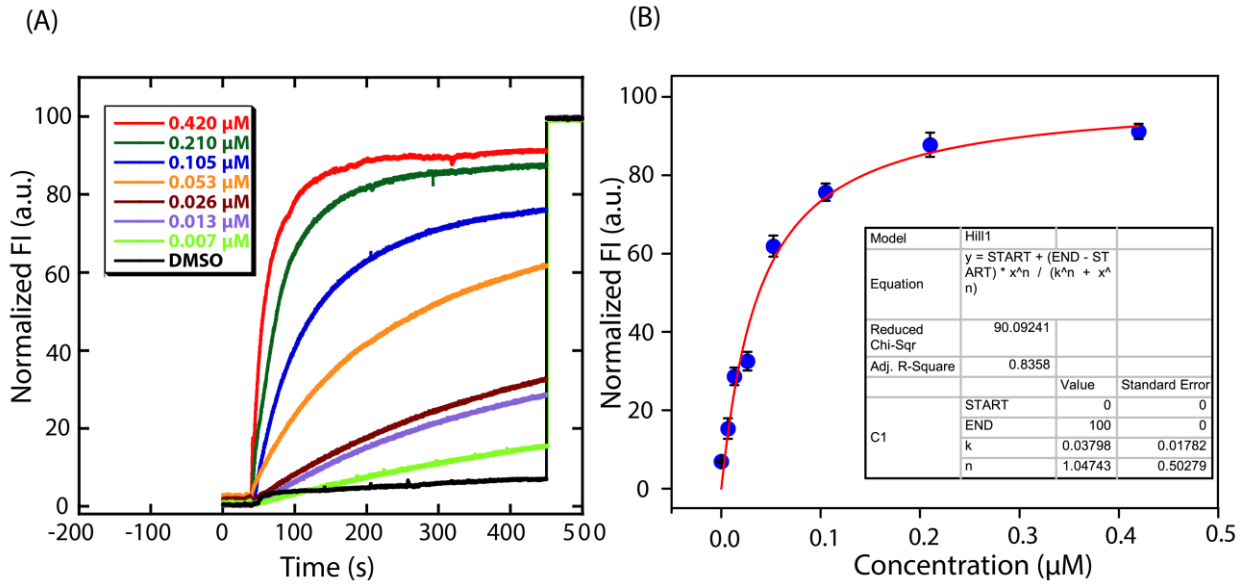


Fig. S8. Concentration-dependent transmembrane transport of Cl^- ion in the presence of compound **1c** across the EYPC/CHOL-LUV⊃HPTS. The ion transport activity was measured by HPTS fluorescence assay using a pH gradient (pH_{in} = 7.2, pH_{out} = 7.8; A). The EC_{50} value was calculated using the modified Hill equation (B).

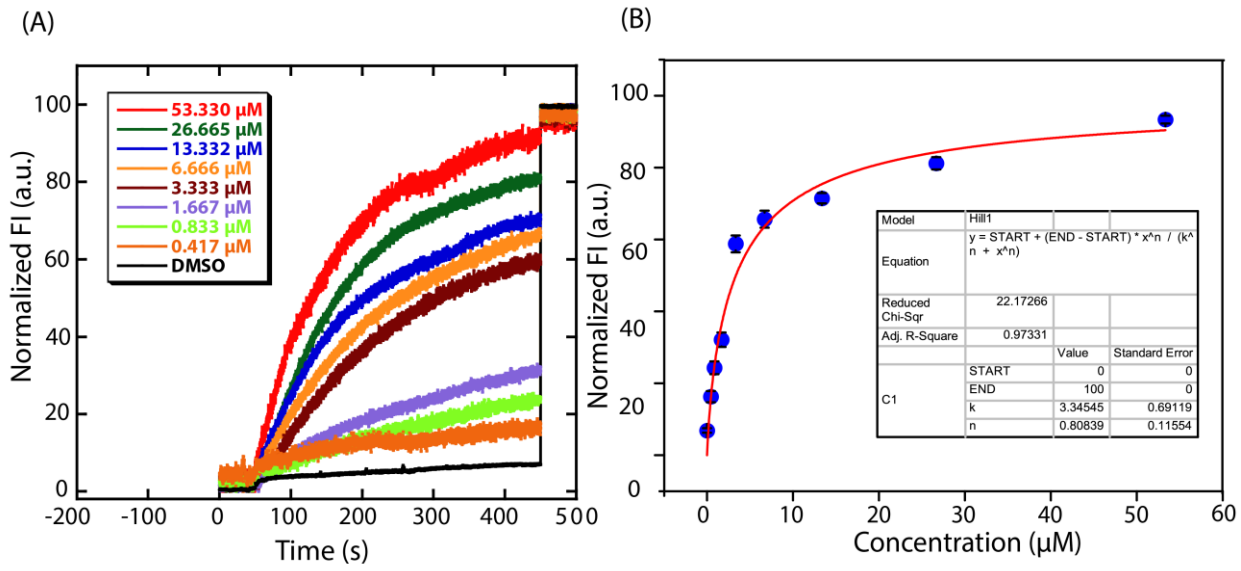


Fig. S9. Concentration-dependent transmembrane transport of Cl^- ion in the presence of compound **1d** across the EYPC/CHOL-LUV⊃HPTS. The ion transport activity was measured by HPTS fluorescence assay using a pH gradient (pH_{in} = 7.2, pH_{out} = 7.8; A). The EC_{50} value was calculated using the modified Hill equation (B).

4. Ion Selectivity Studies.

4.1. ***Buffer and stock solution preparation*** **—** Requisite amount of HEPES and MCl or Na_xA salt (LiCl, NaCl, KCl, RbCl, CsCl, NaBr, NaI, $NaNO_3$ and NaOAc) were added and dissolved in Milli-Q water to attain a final concentration of 20 mM HEPES and 100 mM of the respective salt.

4.2. ***Cation selectivity studies*** **—**The HPTS fluorescence assay was done by adding 2890 µL of buffer solution (20 mM HEPES buffer, pH 7.2, containing 100 mM of MCl salt), 50 µL of EYPC/CHOL-LUV⊃HPTS and 50 µL of 0.5 M NaOH in a clean and dry 3 mL fluorescence cuvette placed in the fluorescence spectrophotometer (Fluoromax-4 spectrofluorometer) at room

temperature under the slow stirring condition for around 3 minutes. During this time, a pH gradient of ~ 0.6 between the extra- and intravesicular systems was generated. The HPTS fluorescence intensity was monitored (at t = 0 s) at λ_{em} = 510 nm (λ_{ex} = 450 nm). The kinetics was initiated at t = 50 s by adding 10 µL of the respective compound from their DMSO stock solution. The vesicles were lysed at t = 450 s by adding 20 µL 20% Triton X-100 solution, and the fluorescence measurement was continued for an additional 50 s (t = 500 s).

4.3. ***Anion selectivity studies*** **—** Similar procedure was followed according to the earlier section except that here 20 mM HEPES buffer, pH 7.2 containing 100 mM Na_xA solutions were used where A = Cl^-, Br^-, I^-, F^-, and SCN^-.

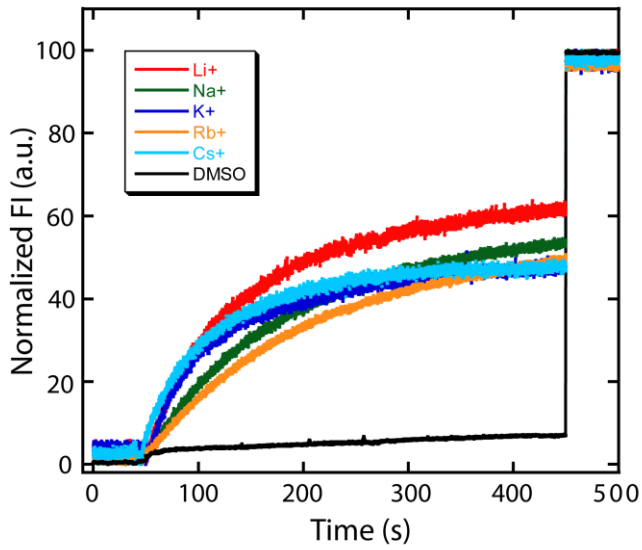


Fig. S10. Cation selectivity of compound **1c** (0.038 µM)**,** as measured by HPTS assay in the presence of pH gradient.

5. Evidence for the mechanistic pathway for chloride ion transport.

5.1. ***Ion transport activity in the presence of FCCP (FCCP assay)*** **—**The vesicles were prepared by following a similar procedure, as discussed in earlier section 3.1. The ion transport activity was measured in the presence as well as in the absence of FCCP dye. The HPTS fluorescence assay was done by adding 2890 µL of buffer solution (20 mM HEPES buffer, pH 7.2, containing 100 mM NaCl), 50 µL of EYPC/CHOL-LUV⊃HPTS and 50 µL of 0.5 M NaOH in a clean and dry 3 mL fluorescence cuvette placed in the fluorescence spectrophotometer (Fluoromax-4 spectrofluorometer) at room temperature under the slow stirring condition for around 3 minutes. During this time, a pH gradient of ~ 0.6 between the extra- and intravesicular systems was generated. The HPTS fluorescence intensity was monitored (at t = 0 s) at λ_{em} = 510 nm (λ_{ex} = 450 nm). The kinetics was initiated at t = 50 s by adding 10 µL of a solution containing 8 µL compound and 2 µL FCCP from their respective DMSO stock solution. The vesicles were lysed at t = 450 s by adding 20 µL 20% Triton X-100 solution, and the fluorescence measurement was continued for an additional 50 s (t = 500 s). A similar experiment was carried out by adding 10 µL of the compound solution only without FCCP at t = 50 s.

5.2. ***Ion transport activity in the presence of valinomycin*** **—** The vesicles were prepared by following a similar procedure, as discussed in earlier section 3.1. The HPTS fluorescence assay was done by adding 2890 µL of buffer solution (20 mM HEPES buffer, pH 7.2, containing 100 mM NaCl), 50 µL of EYPC/CHOL-LUV⊃HPTS and 50 µL of 0.5 M NaOH in a clean and dry 3 mL fluorescence cuvette placed in the fluorescence spectrophotometer (Fluoromax-4 spectrofluorometer) at room temperature under the slow stirring condition for around 3 minutes. During this time, a pH gradient of ~ 0.6 between the extra- and intravesicular systems was generated. The HPTS fluorescence intensity was monitored (at t = 0 s) at λ_{em} = 510 nm (λ_{ex} = 450

nm). The kinetics was initiated at t = 50 s by adding 10 µL of a solution containing 8 µL compound and 2 µL valinomycin from their respective DMSO stock solution. The vesicles were lysed at t = 450 s by adding 20 µL 20% Triton X-100 solution, and the fluorescence measurement was continued for an additional 50 s (t = 500 s). A similar experiment was carried out by adding 10 µL of the compound solution only without valinomycin at t = 50 s.

5.3. ***Evidence for mobile carrier mechanism.***

5.3.1. ***Cholesterol dependency assay*** **—** The LUVs were prepared in a similar manner as described in the earlier section, only the molar ratios of EYPC and cholesterol were varied as 10:0, 8:2 and 6:4 to explore the effect of cholesterol on Cl^- ion transport activity of the compounds. Increasing the concentration of cholesterol is known to slow the diffusion process, which will be more prominent in the case of a mobile carrier mechanism. At the same time, not much significant difference is expected in the case of an ion channel mechanistic pathway. A similar experimental procedure was followed, as mentioned in the earlier section, except that here, for each experiment, a different liposome solution was used, having a different concentration of cholesterol.

5.4.2. ***U-tube experiment*** **—** confirms the carrier mechanistic path of Cl^- ion transport by **1c**; the classical U-tube experiment was performed. In this experiment, bulk, 20 mL nitrobenzene was taken in the middle of U-tube to mimic the lipid bilayer containing 2 mM of **1c** and 2 mM tetrabutylammoniumhexafluorophosphate. The source end (left side) was filled with 10 mL of 489 mM NaCl solution buffered to pH 7.2 with 5 mM sodium phosphate salts, and the receiver end (right side) was filled with 10 mL of 489 mM $NaNO_3$ solution buffered to pH 7.2 with 5 mM sodium phosphate salts. The organic phase was stirred gently using a magnetic stirrer, and the Cl^-

ion concentration was monitored from time to time using a chloride ion-selective electrode for 72 hours.

5.5. ***Test for the leaching-out of the compounds from the membrane bilayer environment*** **—** The assay was performed to prove the membrane bilayer location of the compounds over the course of the different experiments being performed. If the compounds are in the membrane bilayer, then with the dilution of the lipid solution, the transport rate should remain the same. For this assay, various concentrations of EYPC/CHOL-LUV⊃HPTS in 20 mM HEPES buffer at pH 7.2, containing 100 mM NaCl (final concentration of the vesicles were 300 µM, 400 µM, 500 µM, and 600 µM) were taken in a 3 mL fluorescence cuvette followed by addition of the compound **1c** (10 µL from the stock solution to maintain a fixed ionophore/lipid ratio in all cases). The cuvette was then placed under the stirring conditions for maximum incorporation of the compounds into the lipid bilayer. The HPTS fluorescence intensity was monitored (at t = 0 s) at λ_{em} = 510 nm (λ_{ex} = 450 nm). The kinetics was initiated at t = 50 s by adding 10 µL of the compound solution **1c** from the respective DMSO stock solution. The vesicles were lysed at t = 450 s by adding 20 µL 20% Triton X-100 solution, and the fluorescence measurement was continued for an additional 50 s (t = 500 s). Four lipid concentrations were used for this assay, and the transport efficacy was found to be the same in all these four cases suggesting that the compounds were in the lipid bilayer during the course of all the experiments.

6. Regeneration of the active transporter by dealkylation of the proanionophore.

6.1. ***Regeneration by using GSH*** **—** The bromosulfonium salt of compound **2** (1 mM) was dissolved in methanol, and 10 mM PBS, pH 7.4, containing 2 mM reduced glutathione GSH, and

the mixture was incubated at 37 °C. At different time points, an aliquot of the reaction solution was removed and monitored by HPLC analysis. The aliquots were dissolved in methanol before its injection to the analytical HPLC (Ultimate 3000, Thermo Fisher Scientific) system. The Hypersil GOLD™ C18 Selectivity LC Column and a Charged Aerosol Detector (CAD) were used for the analysis. Methanol/water gradient (95% methanol and 5% water) was used as the mobile phase at a flow rate of 0.5 mL/minute for 25 minutes run time.

6.2. ***Regeneration by using Fenton's reagent*** ─ The bromosulfonium salt of compound **2** (1mM) was dissolved in methanol, and 10 mM PBS, pH 7.4 containing Fenton's reagent (1 mM Fe^{2+} and 5 mM H_2O_2). The mixture was then incubated at 37 °C. At different time points, an aliquot of the reaction solution was collected, and the dealkylation efficiency was monitored by HPLC analysis.

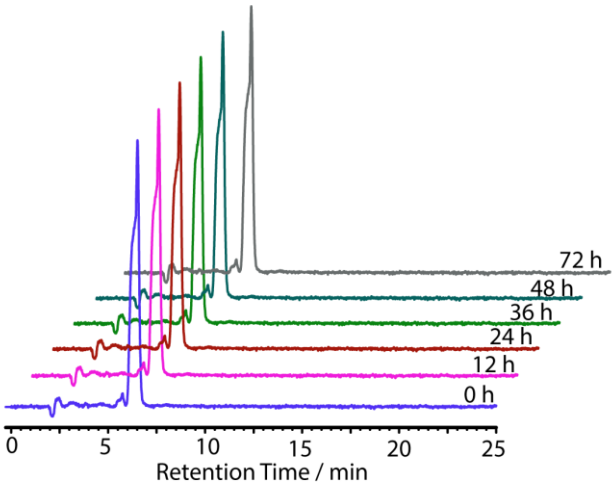


Fig. S11. HPLC traces of proanionophore **2** at different time intervals in the absence of Fenton's reagent. A similar spectral pattern was observed in the absence of GSH.

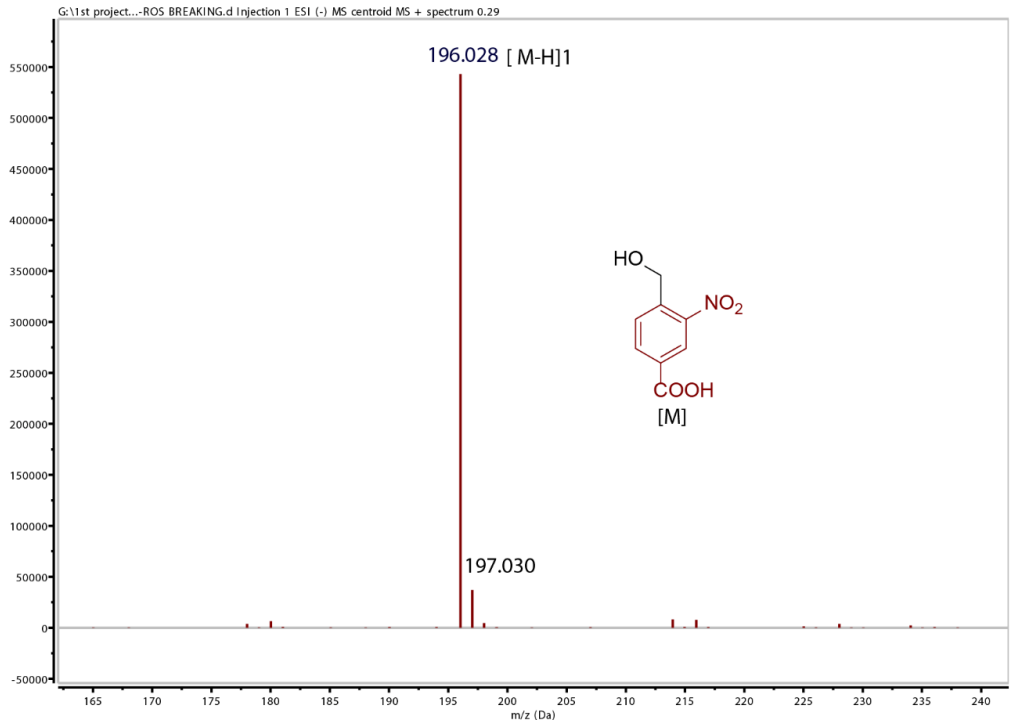


Fig. S12. Mass spectra of the intermediate **3** generated due to the cleavage of proanionophore **2** in the presence of Fenton's reagent.

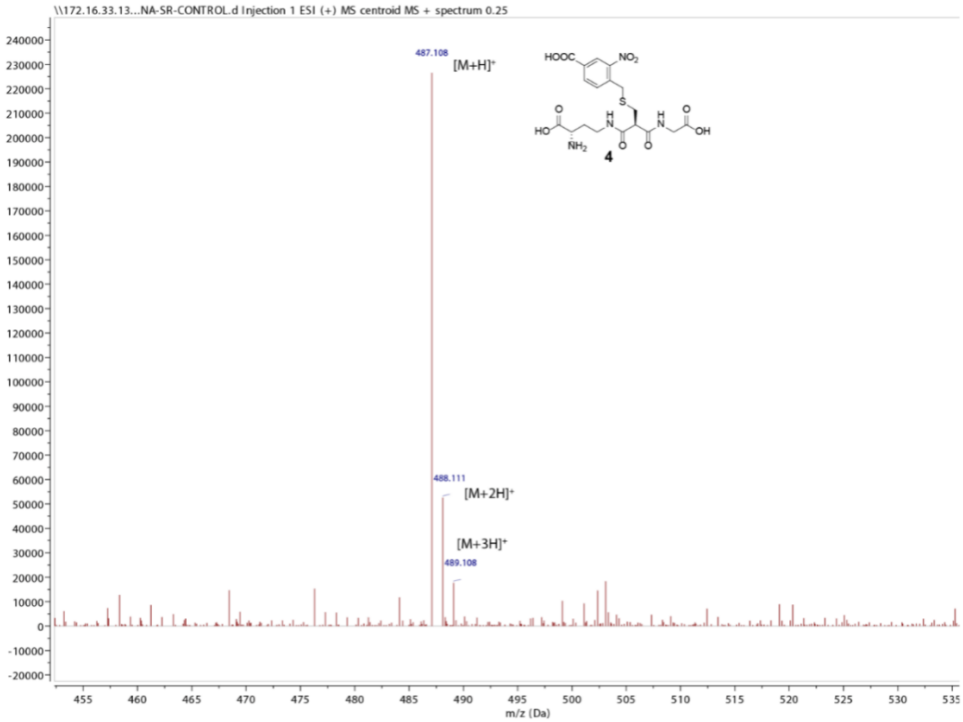


Fig. S13. Mass spectra of the glutathione derivative **4**.

6.3. ***Photolysis Studies*** **—** In a clean and dry NMR tube, the solution of proanionophore **2** (5 mg in 0.5 mL) was taken in DMSO-d6. The ^{1}H NMR spectrum of the sample was recorded first (t = 0 min). Then, the tube was kept in a photoreactor and irradiated with UV light (3×3 Watt LEDs, λ = 365 nm) for 10 min, and ^{1}H NMR spectrum of the irradiated sample was recorded. The ^{1}H NMR spectra were processed using MestReNova 6.0 by considering residual solvent peak as an internal reference. We have also performed the FT-IR experiment, the sulfonium salt **2** was dissolved in methanol, and it was kept in a photoreactor and irradiated with UV light for 10 min, and the FT-IR spectrum was recorded. Therefore, upon photoirradiation, the appearance of the new aldehyde proton peak signal was generated, and the photolytic conversion of proanionophore **2** to carrier **1c** was confirmed by ^{1}H NMR and FT-IR spectrum.

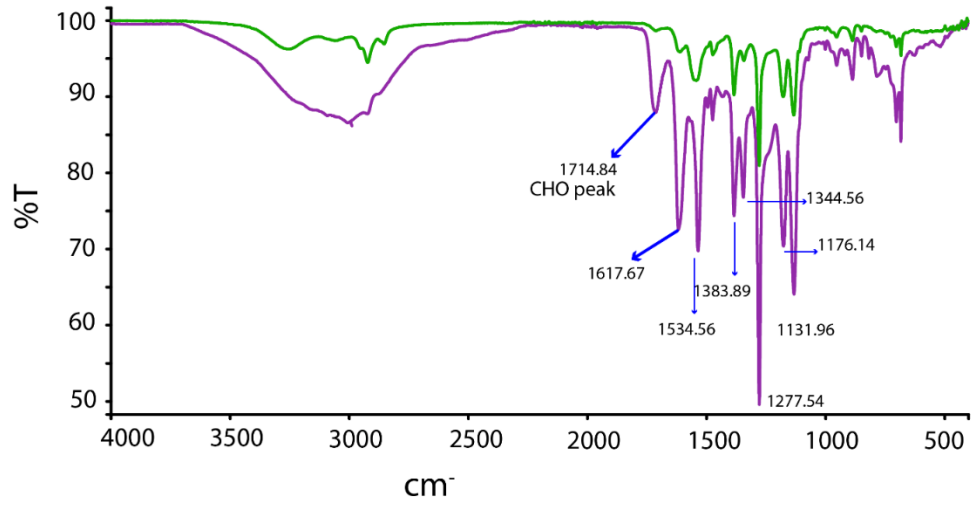


Fig. S14. FT-IR spectrum of sulfonium salt **2** in the absence of 365 nm UV light (green line) and after 10 min (violet line) irradiated with 365 nm light.

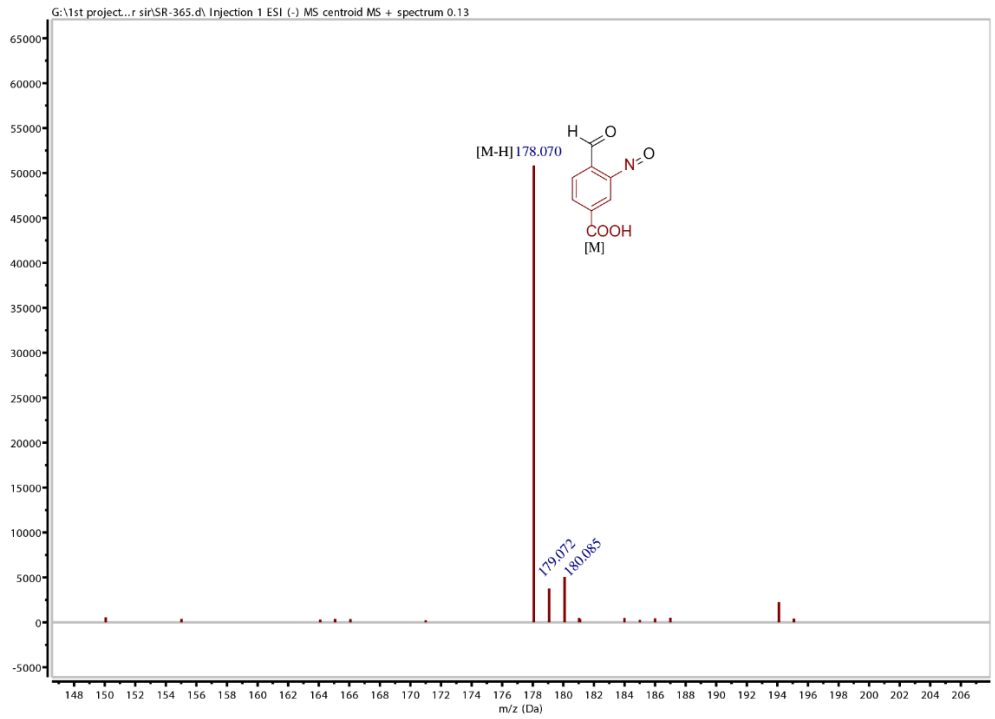


Fig. S15. Mass spectra of intermediate **5** upon photoirradiation of compound **2**.

7. Ion transport activity of regenerated anionophore by fluorescence-based assay.

7.1. ***Ion transport activity of glutathione mediated regenerated anionophore*** **—** The bromosulfonium salt **2** (1 mM) was dissolved in methanol, and 10 mM PBS, pH 7.4, containing 2 mM reduced GSH, and the mixture was incubated at 37 °C. At different time points (0 h, 12 h, 24 h, 36 h, 48 h, 72 h), an aliquot of the reaction solution was removed. Using similar fluorescence-based assay as reported in the earlier section, the Cl^- ion transport efficiency of the aliquots was tested. A control experiment was done by adding DMSO instead of bromosulfonium salt **2**, and similarly, the aliquots were tested.

7.2. ***Fenton's reagent mediated regeneration of carrier from proaninophore*** **—** Regeneration by using the bromosulfonium salt **2** (1 mM) was dissolved in methanol and 10 mM PBS, pH 7.4 containing Fenton's reagent (1 mM Fe^{2+} and 5 mM H_2O_2) and the mixture was incubated in 37 °C.

At different time points (0h, 2h, 4h, 6h, 8h, 12h), an aliquot of the reaction mixture was removed, and the Cl^- ion efficiency was analyzed by the fluorescence-based method as reported in the earlier section.

7.3. ***Light mediated regeneration of carrier from proaninophore*** **—** In a clean and dry 3 mL fluorescence cuvette 2890 µL of buffer solution (20 mM HEPES buffer, pH 7.2, containing 100 mM NaCl), 50 µL of EYPC/CHOL-LUV⊃HPTS and 50 µL of 0.5 M NaOH were taken and placed in the fluorescence spectrophotometer (Fluoromax-4 spectrofluorometer) at room temperature under the slow stirring condition for around 3 minutes. During this time, a pH gradient of ~ 0.6 between the extra- and intravesicular systems was generated. While in a clean glass sample vial, 63 µM of the proaninophore **2** was taken and irradiated with UV light (3 × 3 Watt LEDs, λ = 365 nm) for 5 minutes and 10 minutes and the two aliquots were taken. The HPTS fluorescence intensity was monitored (at t = 0 s) at λ_{em} = 510 nm (λ_{ex} = 450 nm). The kinetics was initiated at t = 50 s by adding 10 µL of the respective aliquots. The vesicles were lysed at t = 450 s by adding 20 µL 20% Triton X-100 solution, and the fluorescence measurement was continued for an additional 50 s (t = 500 s).

8. NMR spectra of the synthesized compounds.

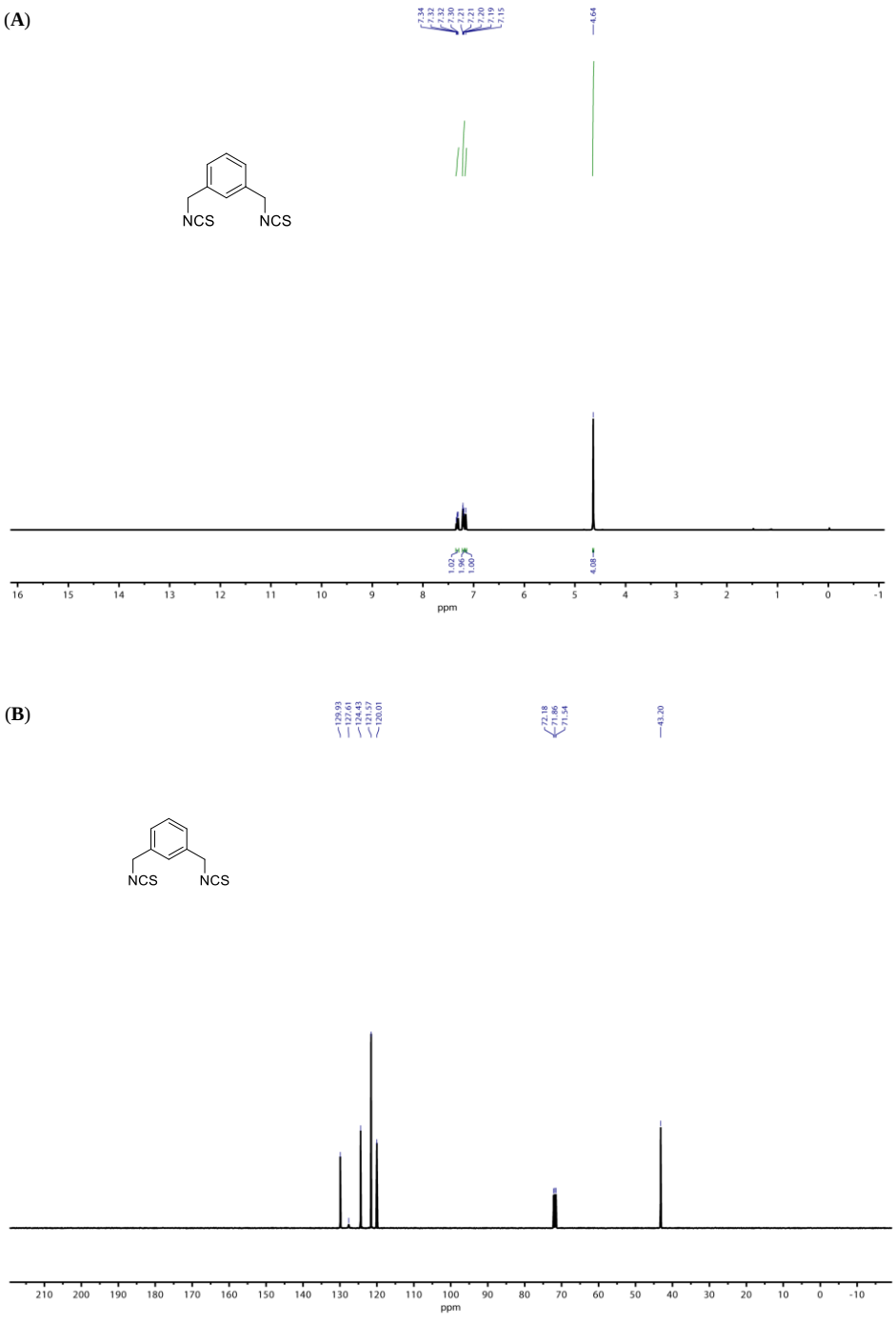


Fig. S16. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of 1,3-bis(isothiocyanatomethyl)benzene in the $CDCl_3$ solvent.

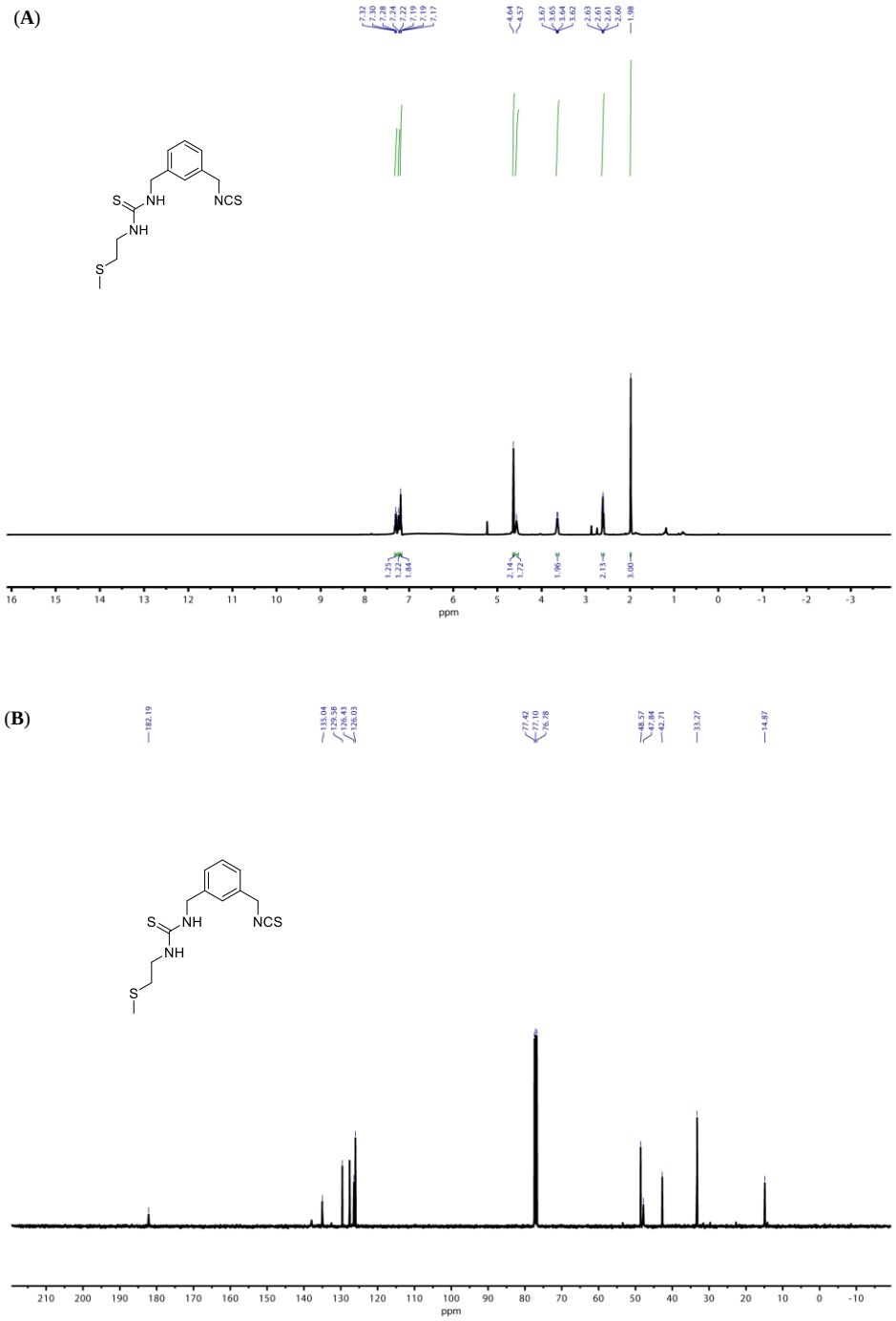


Fig. S17. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound 1-(3-(isothiocyanatomethyl)benzyl)-3-(2-(methylthio)ethyl)thiourea in the $CDCl_3$ solvent.

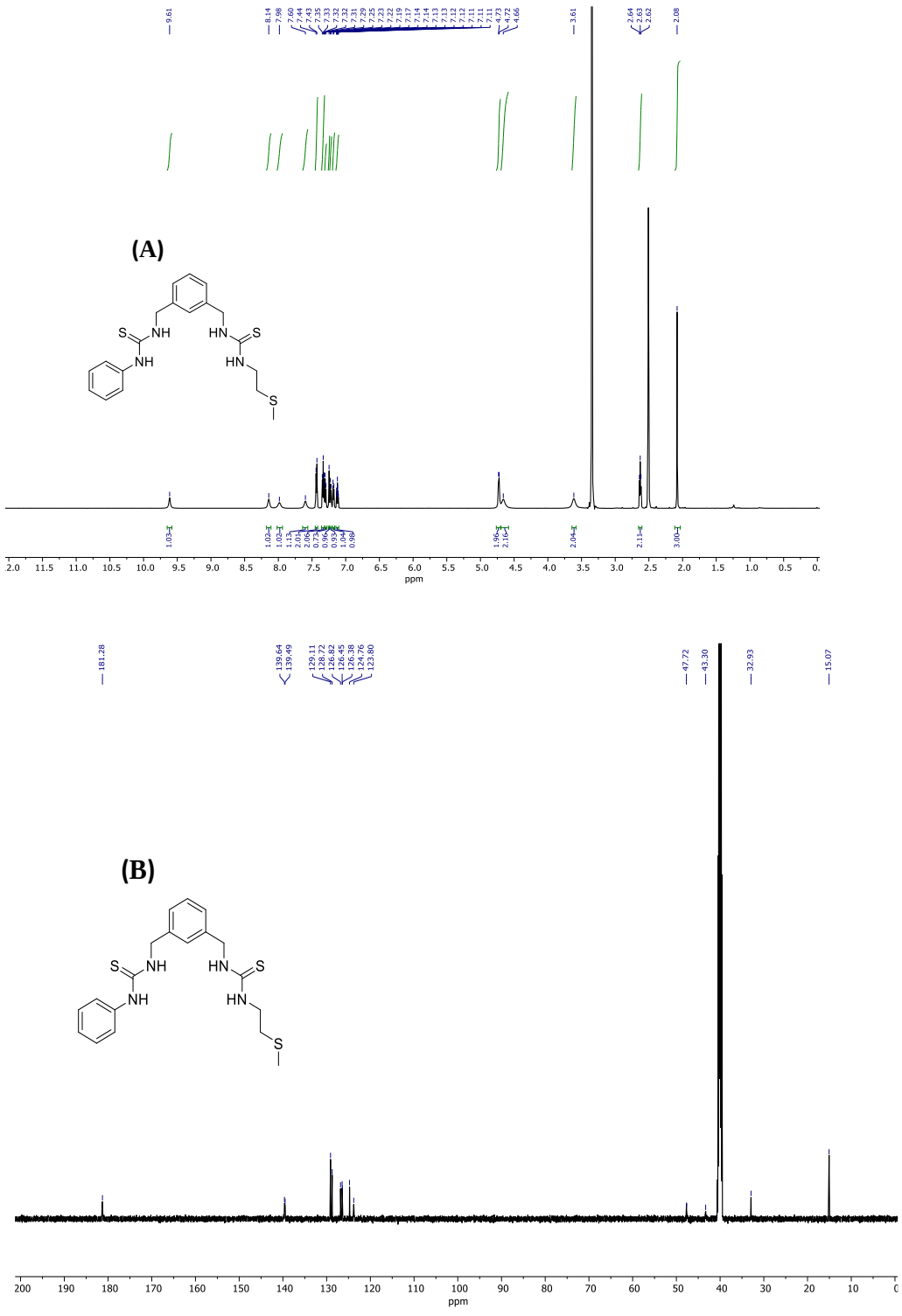


Fig. S18. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of compound **1a** in the $CDCl_3$ solvent.

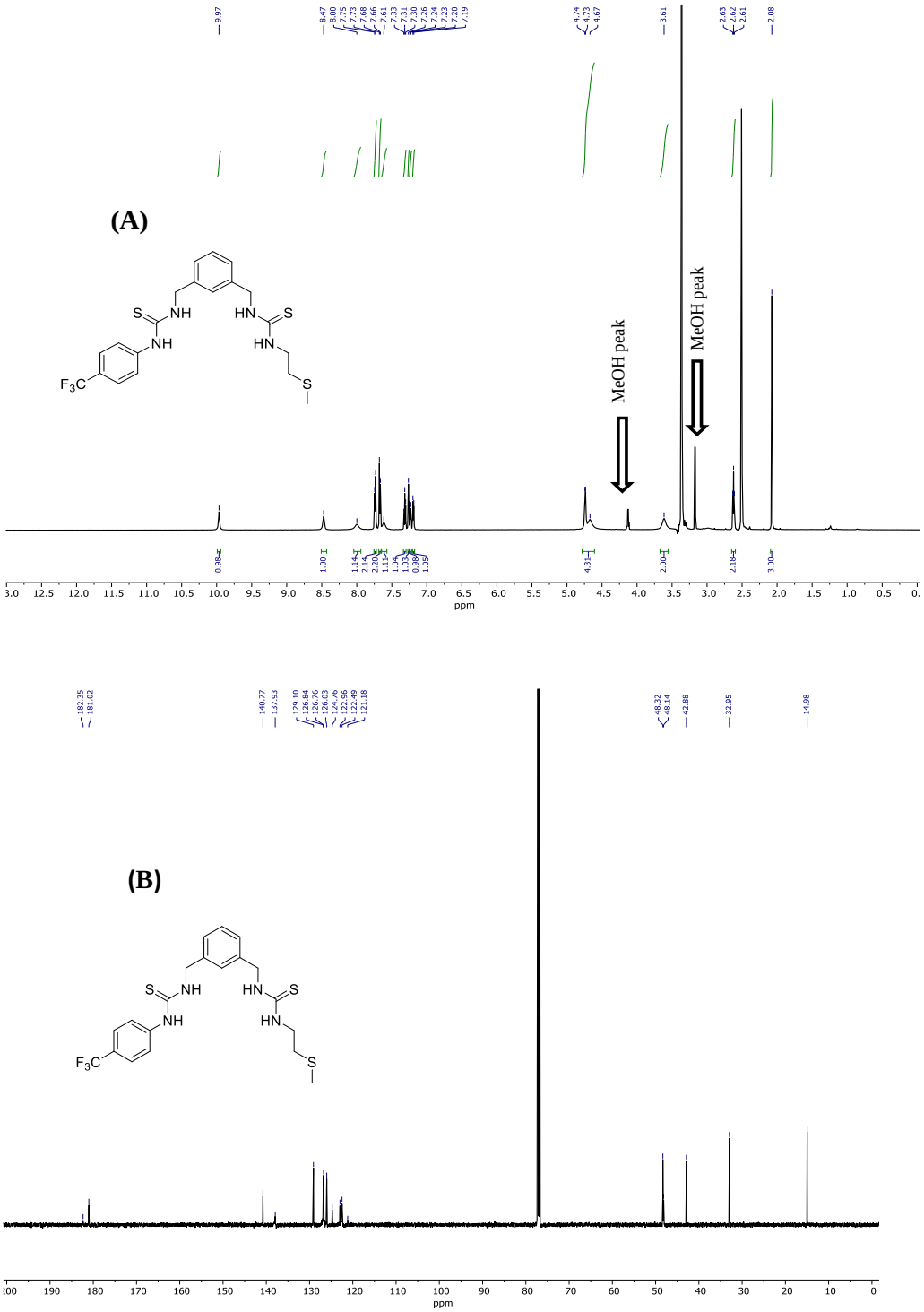


Fig. S19. ^{1}H NMR (A) in DMSO-$d_6$and ^{13}C (B) spectra of compound **1b** in the $CDCl_3$ solvent.

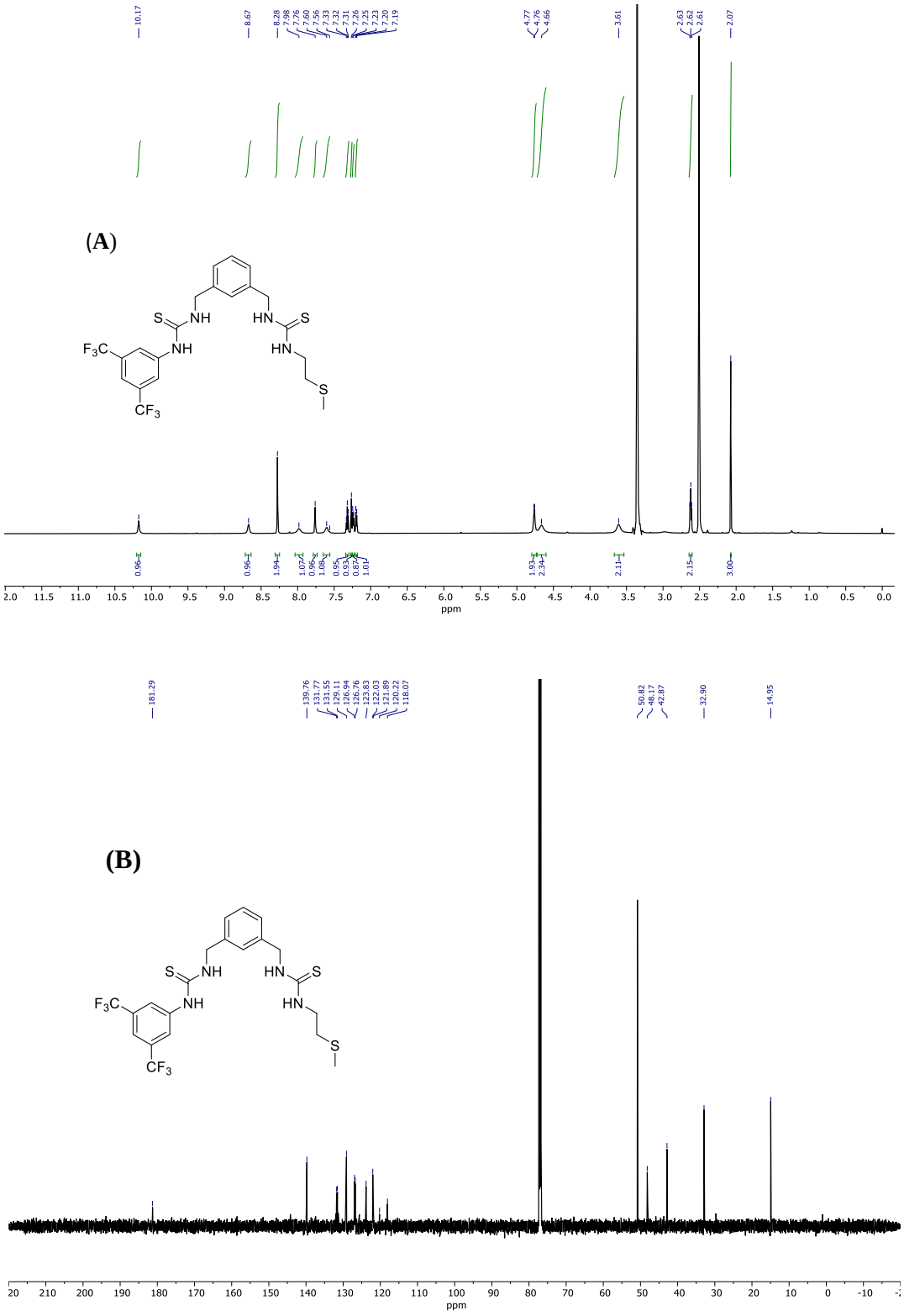


Fig. S20. ^{1}H NMR (A) in DMSO-$d_6$and ^{13}C (B) spectra of compound **1c** in the $CDCl_3$ solvent.

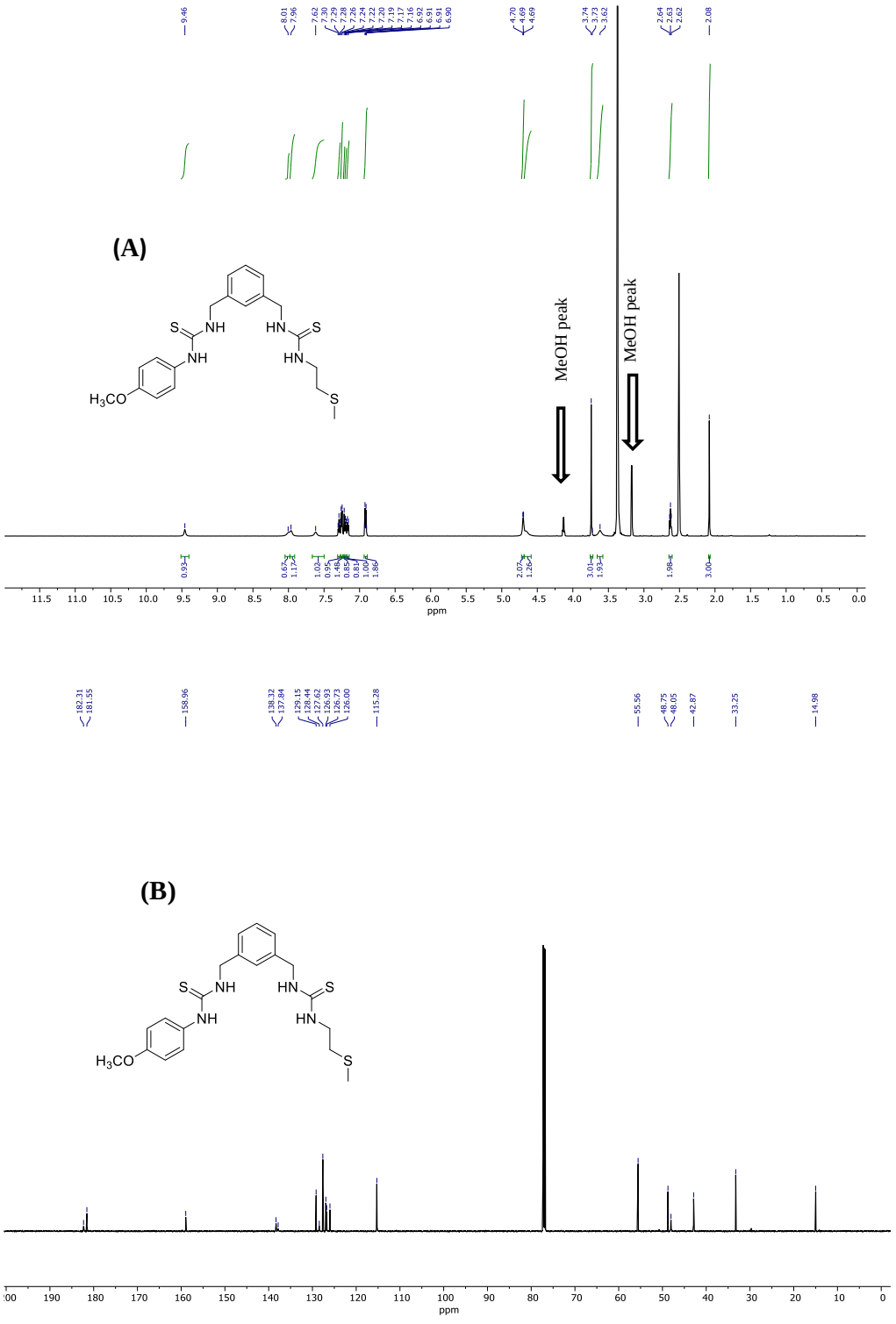


Fig. S21. ^{1}H NMR (A) in DMSO-d_6 and ^{13}C NMR (B) spectra of compound **1d** in the $CDCl_3$ solvent.

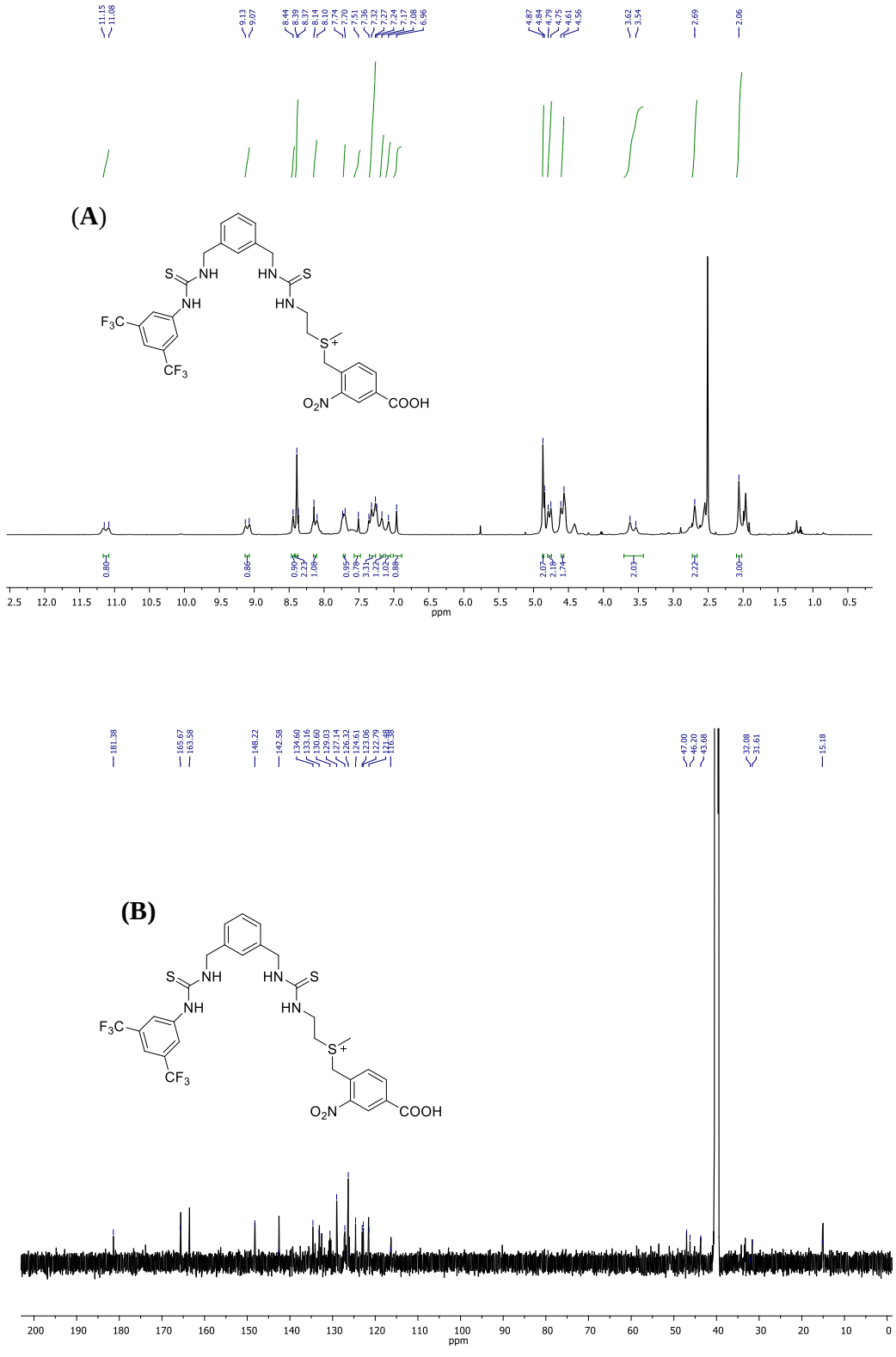


Fig. S22. ^{1}H NMR (A) and ^{13}C (B) spectra of compound **2** in the DMSO-d_6 solvent.