SUPPORTING INFORMATION

Stimuli-responsive release of active anionophore from RGD-peptide-linked proanionophore

Soumya Srimayee,[a] Saumya Ranjan Badajena,[b] Nasim Akhtar,[a] Mrinal Kanti Kar[a] Subhasis Dey,[a] Purusottam Mohapatra*[b] and Debasis Manna*[a]

[a]Department of Chemistry, Indian Institute of Technology Guwahati, Assam-781039, India.
[b]Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER), Guwahati, Changsari, Assam-781101, India.
Email: dmanna@iitg.ac.in

1. General methods

All chemical reactions were performed under an inert atmosphere. All reagents and solvents for synthesis were purchased from commercial sources (Sigma Aldrich, TCI) and used further without purification. The column chromatography was carried out using silica (100−200 mesh size). The thin-layer chromatography was performed on silica gel 60−F254 plates. Egg yolk phosphatidylcholine (EYPC) as a solution of chloroform (25 mg/ mL), mini extruder, and polycarbonate membrane of 100 nm and 200 nm were purchased from Avanti Polar Lipid. Cholesterol, HEPES, HPTS, lucigenin, Triton X-100, valinomycin, DMSO, and all inorganic salts were obtained as molecular biology grade from Sigma Aldrich. The ^{1}H NMR spectra were recorded at 500 MHz, whereas ^{13}C NMR was recorded at 500 MHz. The residual solvent signals were considered as an internal reference (^{1}H NMR $CDCl_3$: δ_{ppm} 7.26 ppm; ^{13}C NMR $CDCl_3$: δ_{ppm} 77.2 ppm; ^{1}H NMR DMSO-d_6: δ_{ppm} 2.54 ppm; ^{13}C NMR DMSO-d_6: δ_{ppm} 39.5 ppm) to calibrate spectra. The chemical shifts were reported in ppm. The following abbreviations were used to

indicate multiplicity patterns m: multiplet, s: singlet, d: doublet, t: triplet, q: quartet, dd: doublet of doublet, ddd: doublet of doublet of doublet, td: triplet of doublet, dt: doublet of triplet. Coupling constants were measured in Hz. High−resolution mass spectra (HRMS) were recorded on electrospray ionization time−of−flight (ESI−TOF). Fluorescence experiments were recorded on Fluoromax−4 from HORIBA equipped with an injector port and magnetic stirrer in a microfluorescence cuvette. All buffer solutions were prepared from the autoclaved water. The pH of buffer solutions was adjusted using the Helmer pH meter. The extravesicular dye was removed by performing gel chromatography using Sephadex G-50. The fluorescence studies were conducted using Origin 6.0.

2. Estimation of pK_a and logP values

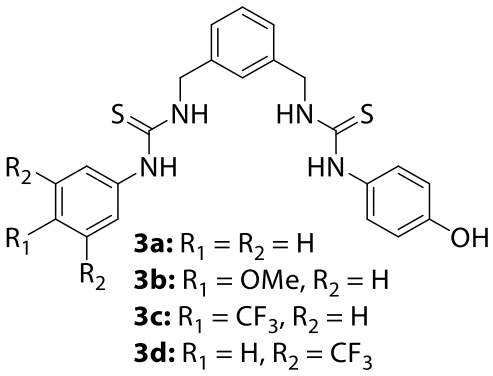
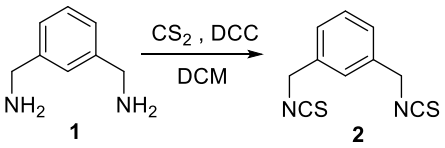


Fig. S1. Structure of the synthesized ionophores **3a-3d**.

Table S1. pK_a and logP of compound **3a-3d**.

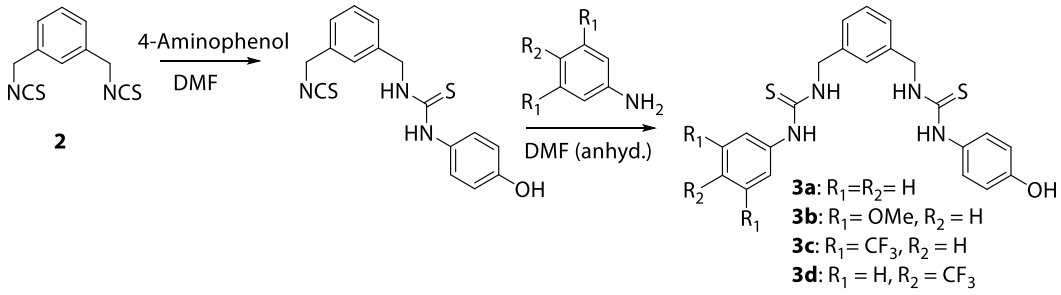
compound	R_1	R_2	pK_a	clogP
3a	H	H	9.606	2.489
3b	OMe	H	9.606	2.592
3c	CF_3	H	9.606	3.901
3d	H	CF_3	9.606	4.969

3. Synthesis and characterization of the compounds



Scheme S1. Synthesis of compound 1,3-bis(isothiocyanatomethyl)benzene (**2**).

3.1. ***Synthesis of 1,3-bis(isothiocyanatomethyl)benzene (2)*** **—** To the stirring solution of 1,3-phenylenedimethanamine (400 mg, 2.93 mmol) and *N*, *N*′-dicyclohexylcarbodiimide (2.231 g, 29.3 mmol) in dry CH_2Cl_2, CS_2 (1.211 g, 5.87 mmol) was added (dropwise) under the continuous stirring condition at 0 °C. Then the reaction mixture was stirred at room temperature until the maximum consumption of the starting material. The progress of the reaction was monitored by the TLC method (14% ethyl acetate/hexane). The residue was filtered out and concentrated under reduced pressure, which was further purified by a silica-gel-based column chromatographic technique using a 0-8% ethyl acetate/hexane system to obtain a yellowish liquid product with a 70% yield. The compound was characterized with ^{1}H and ^{13}C NMR, and the characteristic peaks are in accordance with the reported ones.[1]



Scheme S2. Synthetic of compound **3a-3d**.

3.2. ***General method for preparation of bis-thiourea compound*** **—** To the stirring solution of compound **2** (0.6 mmol) in DMF, 4-aminophenol (0.48 mmol) was added and stirred for 4h at room temperature. Then respective amines (0.6 mmol) in DMF were added to the mixture and stirred until maximum consumption of the starting materials. Then it was extracted using ethyl acetate and chilled ice water (3 × 15 mL); layers were combined, passed over anhydrous sodium sulfate, and concentrated under reduced pressure.

3.3. ***Synthesis of 1-(3-((3-(4-hydroxyphenyl) thioureido) methyl) benzyl)-3-phenylthiourea (3a)*** **—** Compound **2** (50 mg, 0.1 mmol) and aniline (10 mg, 0.12 mmol) were used in the reaction following general protocol mentioned above. The crude reaction mixture was purified through column chromatography with a solvent gradient system of CH_2Cl_2/MeOH (0-5%) to obtain a white solid compound with a 66% yield. Characterization: **^{1}H NMR** (500 MHz, DMSO d_6) δ_{ppm} 9.58 (s, 1H), 9.38 (s, 1H), 9.29 (s, 1H), 8.13 (s, 1H), 7.80 (s, 1H), 7.44 (d, J = 7.6 Hz, 2H), 7.31 (m, 3H), 7.27 (s, 1H), 7.21 (t, J = 7.8 Hz, 2H), 7.11 (t, J = 9.3 Hz, 3H), 6.73 (d, J = 8.2 Hz, 2H), 4.75 – 4.70 (m, 4H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ_{ppm} 181.6, 181.2, 155.5, 139.9, 139.6, 139.4, 129.1,

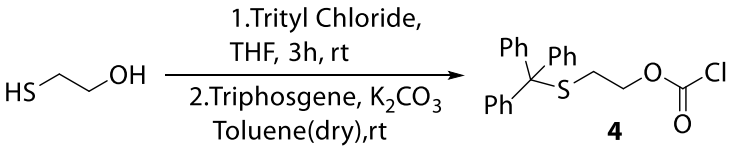
128.6, 127.0, 126.6, 126.3, 124.7, 123.8, 115.8, 47.7. **HRMS**: ESI calc. for $C_{22}H_{22}N_4OS_2$ $[M+H]^+$: 423.1314, found: 423.1316.

3.4. ***Synthesis of 1-(3-((3-(4-hydroxyphenyl) thioureido) methyl) benzyl)-3-(4-methoxyphenyl) thiourea (3b)*** **—** Compound **2** (50 mg, 0.1 mmol) and 4-methoxyaniline (13 mg, 0.1 mmol) were set in the reaction following general protocol mentioned above. The crude reaction mixture was purified through column chromatography with a solvent gradient system of CH_2Cl_2/MeOH (0-5%) to give a solid white product with a 90% yield. Characterization: **^{1}H NMR** (600 MHz, DMSO-d_6) δ_{ppm} 9.43 (s, 1H), 9.41 (s, 1H), 9.32 (s, 1H), 7.92 (s, 1H), 7.81 (s, 1H), 7.28 (t, J = 7.6 Hz, 1H), 7.26 (s, 1H), 7.24 (d, J = 4.8 Hz, 2H), 7.18 (s, 2H), 7.10 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 6.73 (d, J = 8.6 Hz, 2H), 4.71 (d, J = 3.4 Hz, 4H), 3.73 (s, 3H). **^{13}C NMR** (150 MHz, DMSO-d_6) δ_{ppm} 181.5, 157.1, 155.5, 139.8, 131.9, 128.5, 127.1, 126.4, 126.2, 115.8, 114.5, 55.6, 47.7. **HRMS**: ESI calc. for $C_{23}H_{24}N_4O_2S_2$ $[M+H]^+$: 453.1418, found 453.1421.

3.5. ***Synthesis of 1-(3-((3-(4-hydroxyphenyl) thioureido) methyl) benzyl)-3-(4-(trifluoromethyl) phenyl) thiourea (3c)*** **—** Compound **2** (50 mg, 0.1 mmol) and 4-trifluoromethyl aniline (16.28 mg, 0.1 mmol) were set in the reaction following general protocol mentioned above. The crude reaction mixture was purified through column chromatography with a solvent gradient system of 0-5% CH_2Cl_2/MeOH to give a white-solid product with a 78% yield. Characterization: **^{1}H NMR** (600 MHz, DMSO- d_6) δ_{ppm} 9.94 (s, 1H), 9.47 (s, 1H), 9.31 (s, 1H), 8.45 (s, 1H), 7.81 (s, 1H), 7.73 (s, 1H), 7.71 (s, 1H), 7.65 (s, 1H), 7.64 (s, 1H), 7.30 (t, J = 7.6 Hz, 1H), 7.26 (s, 1H), 7.20 (dd, J = 12.0, 7.9 Hz, 2H), 7.08 (d, J = 8.1 Hz, 2H), 6.71 (d, J = 8.3 Hz, 2H), 4.72 (d, J = 20.4 Hz, 4H). **^{13}C NMR** (150 MHz, DMSO- d_6) δ_{ppm} 179.84, 179.41, 153.85, 141.98, 138.31, 137.27, 126.99, 125.43, 124.93, 124.68, 124.46, 121.00, 114.16, 45.95. **HRMS**: ESI calc. for $C_{23}H_{21}F_3N_4OS_2$ $[M+H]^+$: 491.1184, found 491.1186.

3.6. ***Synthesis of 1-(3,5-bis(trifluoromethyl) phenyl)-3-(3-((3- (4-hydroxyphenyl) thioureido) methyl) benzyl) thiourea (3d)*** **—** Compound **2** (50 mg, 0.1 mmol) and 3,5-bis(trifluoromethyl) aniline (23 mg, 0.1 mmol) were set in the reaction following general protocol mentioned above. The crude reaction mixture was purified through column chromatography with a solvent gradient system of 0-5% CH_2Cl_2/MeOH to give a white solid product with a 75% yield. Characterization: **^{1}H NMR** (500 MHz, DMSO- d_6) δ_{ppm} 10.17 (s, 1H), 9.38 (s, 1H), 9.31 (s, 1H), 8.67 (s, 1H), 8.28 (s, 2H), 7.82 (s, 1H), 7.75 (s, 1H), 7.32 (t, J = 7.5 Hz, 1H), 7.28 (s, 1H), 7.23 (t, J = 8.2 Hz, 2H), 7.10 (d, J = 7.0 Hz, 2H), 6.72 (d, J = 7.5 Hz, 2H), 4.75 (d, J = 24.1 Hz, 4H).**^{13}C NMR** (125 MHz,

DMSO- d_6) δ_{ppm} 181.43, 181.26, 155.60, 142.30, 140.12, 138.75, 130.67, 130.45, 128.73, 127.12, 126.76, 126.42, 126.36, 124.61, 122.81, 122.60, 116.72, 115.85, 47.61. **HRMS**: ESI calc. for $C_{24}H_{20}F_6N_4OS_2$ $[M+H]^+$: 559.1046, found 559.1052.



Scheme S3. Synthesis of compound 2-(tritylthio)ethyl carbonochloridate (**4**).

3.7. ***Synthesis of 2-(tritylthio)ethyl carbonochloridate (4)*** **—** Compound **4** was synthesized according to the previously reported protocol. Compound **4** was obtained with around 88% yield. The compound was characterized with ^{1}H and ^{13}C NMR, and the characteristic peaks are in accordance with the reported ones.[2]

3.8. ***Synthesis of 4-(3-(3-((3-(3,5-bis(trifluoromethyl)phenyl) thioureido) methyl) benzyl) thioureido) phenyl(2-mercaptoethyl) carbonate (5)*** **—**To the stirring solution of **3d** (100 mg, 0.207 mmol) in dry THF, K_2CO_3 (17 mg, 0.235 mmol) was added and stirred for 1 h. Then 4 (53.4 mg, 0.239 mmol) was added to it dropwise under ice conditions. The reaction mixture was stirred at room temperature for 4 h till completion (monitored by TLC). Purified using preparative TLC (30% Ethyl acetate/hexane). Characterization: **^{1}H NMR** (600 MHz, $CDCl_3$) δ_{ppm} 8.75 (s, 1H), 8.22 (s, 2H), 7.87 (s, 4H), 7.61 (s, 2H), 7.46 (d, J = 7.7 Hz, 13H), 7.32 (t, J = 7.4 Hz, 12H), 7.27 – 7.24 (m, 8H), 7.12 (d, J = 16.2 Hz, 10H), 4.79 (d, J = 42.0 Hz, 4H), 4.13 – 3.77 (m, 5H), 2.69 – 2.50 (m, 5H), 1.45 – 1.14 (m, 6H). **^{13}C NMR** (150 MHz, $CDCl_3$) δ_{ppm} 181.15, 181.08, 153.15, 149.49, 144.41, 139.63, 132.18, 131.96, 129.56, 128.06, 126.93, 126.28, 123.83, 123.06, 122.52, 122.02, 118.58, 67.17, 67.11, 48.63, 48.43, 30.42. **HRMS**: ESI calc. for $C_{46}H_{38}F_6N_4O_3S_3$ $[M+Na]^+$: 927.1902, found: 927.1904.

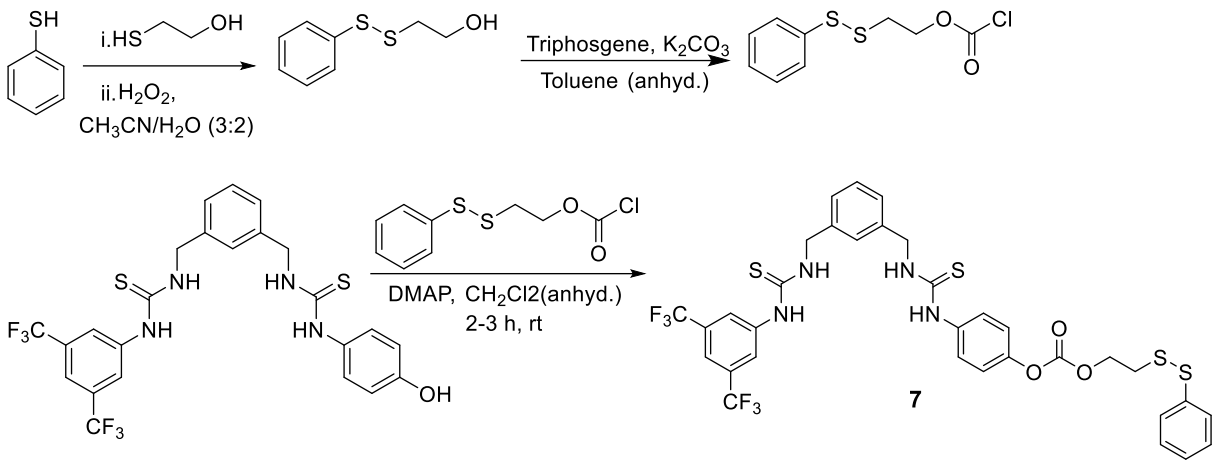
3.9. ***Synthesis of 4-(3-(3-((3-(3,5-bis(trifluoromethyl)phenyl) thiourea) methyl) benzyl) thiourea) phenyl(2-mercaptoethyl) carbonate-CCRCRGDS (6)*** **—** The deprotection of trityl group was carried out under in CH_2Cl_2 by the addition of 30% TFA mixture and reaction was monitored in every 30 min. When the maximum of compound **5** (32 mg, 0.039 mmol) got deprotected, CH_2Cl_2, TFA was evaporated out under high vacuum. To the same pot, acetonitrile/H_2O mixture (4:1), CCRCRGDS peptide (12 mg, 0.013 mmol of **5**) was added and, H_2O_2 was added dropwise under ice condition, and then it was stirred for 10 hours at room

temperature. The reaction was monitored by TLC till starting material get consumed. The solvent was evaporated under a high vacuum, and a solid precipitate gets accumulated. After that, the crude mixture was washed with different solvents (water, CH_2Cl_2, and diethyl ether). Characterization: **^{1}H NMR** (600 MHz, DMSO- d_6) δ_{ppm} 9.81 (s, 1H), 8.75 (s, 1H), 8.25 (s, 1H), 7.71 (s, 4H), 7.36 (s, 1H), 7.17 (s, 3H), 7.03 (s, 1H), 6.76 (t, *J* = 7.6 Hz, 4H), 6.72 (s, 4H), 6.65 (dd, *J* = 14.2, 7.6 Hz, 7H), 6.54 (d, *J* = 8.3 Hz, 4H), 6.17 (d, *J* = 8.3 Hz, 5H), 6.04 (m, 1H), 4.74 (s, 1H), 4.19 (d, *J* = 36.7 Hz, 4H), 3.98 (d, *J* = 7.1 Hz, 2H), 3.92 (t, *J* = 6.3 Hz, 2H), 3.85 (t, *J* = 6.0 Hz, 2H), 3.72 (d, *J* = 7.3 Hz, 2H), 3.11 (t, *J* = 5.6 Hz, 4H), 2.42 (s, 1H), 2.35 (s, 3H), 2.30 (d, *J* = 8.4 Hz, 2H), 2.19 (s, 2H), 2.07 (t, *J* = 6.3 Hz, 1H), 1.99 (s, 4H), 1.28 (s, 2H), 1.14 – 0.86 (m, 5H).**^{13}C NMR** (150 MHz, DMSO- d_6) δ_{ppm} 181.37, 181.21, 172.93, 172.16, 171.22, 170.43, 170.20, 169.60, 163.47, 159.83, 159.62, 159.41, 157.00, 155.30, 142.04, 139.80, 138.75, 131.02, 130.80, 130.58, 130.36, 128.83, 127.22, 126.36, 126.35, 126.28, 124.47, 122.67, 122.62, 120.86, 120.22, 118.25, 116.84, 116.28, 116.09, 115.84, 114.32, 62.08, 54.18, 54.13, 47.44, 47.23, 42.63, 40.72, 40.60, 36.50, 31.39, 29.25, 26.10, 25.79, 25.18, 25.03. **MALDI-TOF** for $[C_{57}H_{76}F_6N_{18}O_{15}S_6]$:1559.7012, found $[M/2]^+$: 779.357.

NOTE: The RGD-peptide sequence (CCRCRGDS) containing three cysteine residues was designed for targeted cargo delivery. Three cysteine residues could install more cargo; however, only one cysteine residue could form a disulfide bond with compound **5.** The reaction of the CCRCRGDS peptide with compound **5** only provided compound **6** with the formation of one disulfide bond. Compound **6** precipitated out of the reaction conditions.

3.10. ***Synthesis of 4-(3-(3-((3-(3,5-bis (trifluoromethyl) phenyl) thioureido) methyl) benzyl) thioureido) phenyl (2-(phenyldisulfaneyl) ethyl) carbonate (7)*** **—** To the stirring solution of 2-mercaptoethanol (354.5 mg, 4.5 mmol) in CH_3CN/H_2O (3:2) mixture, thiophenol (500 mg, 4.5 mmol) was added dropwise. To the above mixture, H_2O_2 (1530 mg,45 mmol) was added and stirred at room temperature for 4 hours (monitored through TLC until completion). Crude mixture 2-(phenyldisulfaneyl) ethan-1-ol was evaporated under reduced pressure and purified in column chromatography (10-20% EA/Hex system). Again, to the stirring solution of K_2CO_3 (446 mg, 3.23 mmol) and triphosgene (479 mg, 1.61 mmol), in anhydrous toluene, 2-(phenyldisulfaneyl) ethan-1-ol was added dropwise in 0 °C. Stirred for another 45 mins, and 2-(phenyldisulfaneyl) ethylcarbonochloridate was obtained in moderate yield (55%). Characterization: **^{1}H NMR (600 MHz, Chloroform-*d*) δ:** 7.57 (d, *J* = 7.2 Hz, 2H), 7.38 (t, *J* = 7.7 Hz, 2H), 7.30 (t, *J* = 7.4 Hz,

1H), 4.54 (t, *J* = 6.7 Hz, 2H), 3.03 (t, *J* = 6.7 Hz, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ:** 150.57, 136.39, 129.26, 128.14, 127.54, 68.93, 35.97.



Scheme S4. Synthesis of control proionophore **7**.

To the stirring solution of **3d**, DMAP was added in anhydrous CH_2Cl_2. Then, to the above solution, 2-(phenyldisulfaneyl)ethylcarbonochloridate was added under 0 ˚C and stirred at room temperature for 2-3 hrs (monitored through TLC). The solvent was evaporated under a rotary evaporator and purified in a column (20-25% EA/Hex system). **^{1}H NMR (600 MHz, Chloroform-*d*) δ:** 8.65 (s, 1H), 8.09 (s, 1H), 7.88 (s, 2H), 7.62 (s, 1H), 7.59 – 7.55 (m, 2H), 7.36 (t, *J* = 7.7 Hz, 2H), 7.28 (d, *J* = 7.6 Hz, 3H), 7.21 – 7.12 (m, 7H), 6.81 (s, 1H), 4.84 (d, *J* = 5.8 Hz, 2H), 4.78 (d, *J* = 5.3 Hz, 2H), 4.49 (t, *J* = 6.6 Hz, 2H), 3.05 (t, *J* = 6.6 Hz, 2H). **^{13}C NMR (151 MHz, $CDCl_3$) δ:** 181.25, 153.27, 149.55, 139.63, 136.54, 132.29, 132.07, 129.30, 129.19, 128.04, 127.39, 127.20, 126.87, 126.54, 125.28, 123.82, 123.23, 122.63, 122.01, 118.67, 66.28, 48.70, 48.48, 36.44. **HRMS**: ESI calculated for $C_{33}H_{28}F_6N_4O_3S_4$, $[M+Na]^+$:793.0841, found 793.0831, , $[M+K]^+$: 809.058, found 809.0574.

(P.S: In the final prepared compound **7**, **^{1}H NMR**: grease peak appeared at – 0.86 ppm and 1.27 ppm. **^{13}C NMR**: grease peak appeared at – 29.7 ppm.)

4. Ion transport studies

4.1. *Ion transport studies with the fluorescence-based assay*

4.1.1. ***buffer and stock solution preparation*** **—** Required buffer solution was prepared by dissolving HEPES and salt (LiCl, NaCl, KCl, RbCl, CsCl, and NaCl, NaBr, NaI, $NaNO_3$) in Milli-Q water to obtain a buffer composition of 20 mM HEPES buffer, pH 7.2, containing 100 mM of the respective salt (MCl or Na_xA). The stock solutions of the compounds were prepared in

molecular biology grade DMSO solvent. The concentration of DMSO stock solution used for our experiment is 0.26% for both ionophore and proionophore.

4.1.2. ***Preparation of EYPC/CHOL-LUVs⊃lucigenin*** **—** For conducting the lucigenin-based ion transport studies, egg yolk phosphatidylcholine (EYPC, 50 mg/mL in deacidified $CHCl_3$) and CHOL (25 mg/mL in deacidified $CHCl_3$) was taken in a clean sample vial in the molar ratio of 8:2. The solution was evaporated by continuous rotation for 6 h under reduced pressure to form a thin lipid film. The thin lipid film was rehydrated by adding 800 µL of 20 mM HEPES buffer containing 1 mM lucigenin and 100 mM $NaNO_3$ solution, pH 7.2. The resultant suspension was vortexed 6-7 times for 1 h, followed by 17-19 freeze-thaw cycles and, finally, 15 minutes of constant vortexing to incorporate lucigenin within the lipid bilayer. The lipid suspension was extruded using a mini extruder (a polycarbonate membrane from Avanti Polar Lipids) with a 200 nm pore size 19-21 times (must be an odd number). The unencapsulated lucigenin dye was removed by size exclusion column chromatography (Sephadex G-50) and 20 mM HEPES buffer containing 1 mM lucigenin and 100 mM $NaNO_3$ solution, pH 7.2 as the eluting solution to get the final lipid concentration of 25 mM (assuming 100% lipid regeneration).

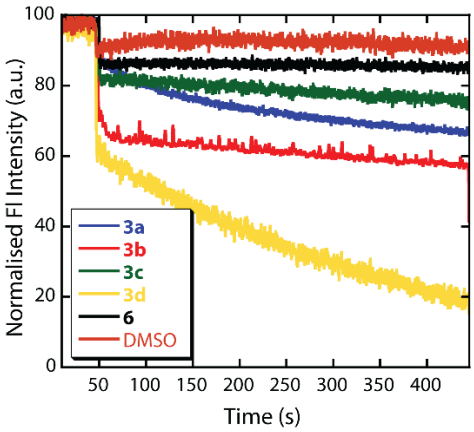


Fig. S2. The Cl^- transport activities of the compounds.

4.1.3 ***Quantitative measurement of transport activity from lucigenin assay*** **—** The fluorescence emission intensities of the lucigenin dye were normalized, and the intensities appearing at t = 0 and t = 500 s were 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 (before the addition of Triton X-100 solution), F_0 = fluorescence intensity immediately before the addition of the transporter (t = 0 s), and F_∞ = fluorescence intensity after the addition of Triton X-100 solution (i.e., at saturation after complete leakage at t = 500 s).

4.2. ***Ion transport activity studies using ion-selective electrode (ISE)-based assay***

4.2.1. ***Chloride ion efflux studies using chloride ion-selective electrode (chloride-ISE)*** **—** The Cl^- ion transport activities of the compounds were measured by monitoring the Cl^- ion concentration outside the liposomes using a chloride ion-selective electrode (chloride-ISE) (Thermo Scientific™ Orion™). Before each experiment, the ISE was calibrated using 1 ppm, 10 ppm, and 100 ppm of standard chloride solution with an ionic strength adjuster solution. A filling solution was poured inside the electrode up to the mark before each experimental session. Chloride concentration (ppm) appearing in the display of the ion meter was set in continuous mode for the time-dependent measurements.

4.2.2. ***Preparation of EYPC/CHOL-LUV*** **—** The LUVs were prepared according to the reported procedure using EYPC and CHOL, as mentioned in the earlier section.[3] The dry film was hydrated with 800 µL of 5 mM Phosphate buffer and 100 mM NaCl. The unilamellar vesicles were dialyzed with 5 mM phosphate buffer, pH 7.2, containing 100 mM $NaNO_3$ to remove the extravesicular NaCl from the solution. Finally, the LUVs were collected, and the volume was adjusted to 800 µL using phosphate buffer (5 mM) and $NaNO_3$ (100 mM) solution. The final lipid concentration was 25 mM (assuming 100% lipid regeneration).

4.2.3 ***Chloride efflux study across EYPC/CHOL-LUV*** **—** To measure the extent of efflux of Cl^- in the absence and presence of the compound, the EYPC/CHOL-LUVs (50 µL) and 5 mM phosphate buffer, pH 7.2, containing 100 mM $NaNO_3$ (3940 µL) were taken in a clean and dry glass vial and kept under mild stirring condition. The glass electrode was immersed into the solution under mild stirring conditions. To initiate the Cl^- transport kinetics at t = 50 s, 10 µL of the respective compound (from DMSO stock solution) was added into the stirring solution, and the readings were noted from the ion meter. After 5 minutes, the vesicles were lysed using 50 µL of 20% Triton X-100 solution. The total Cl^- efflux reading was taken at 7 minutes (allowing

complete disruption of the LUVs). The initial reading was considered 0% Cl─ efflux, and the final reading at 7 min was considered 100% Cl^- efflux.

4.2.4 ***Quantitative measurement of transport activity from chloride ISE assay —*** To find out the EC_{50} value of all the compounds, the chloride-ISE-based Cl^-/NO_3^- exchange assay was performed at varying concentrations. From these experiments, the chloride efflux (%) at 500 s was plotted as a function of the carrier concentration (µM). The data points were fitted to the modified Hill equation using the Origin program 6.0.

$$y = Start + (End - Start) * x\text{^}n/(k^n + x\text{^}n) \quad \ldots\ldots (Eq.S2)$$

In this equation, a = START = control value (DMSO), b = END = 100, y is the chloride efflux efficiency at 500 s (%), and x is the carrier concentration (µM). Where n is the Hill coefficient, EC_{50} values at 500 s can be obtained directly from this modified Hill plot.

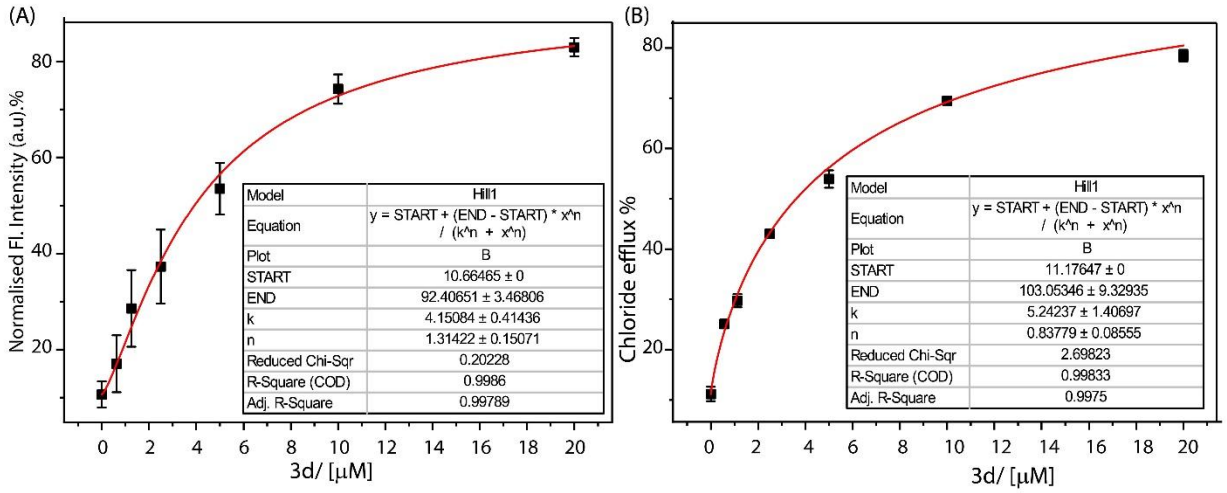


Fig. S3. Concentration-dependent transmembrane transport of Cl^- in the presence of compound **3d** across the LUVs. The ion transport activity was measured by lucigenin-based fluorescence (A) and Cl-ISE (B) assay. The EC_{50} value was calculated using the modified Hill equation.

Note: The standard deviation calculated based on at least 3 experiments is like the following:

FFluorescence-basedEC_{50} value: 4.15 ± 0.82 µM (Hill coefficient, n = 0.78)

ISE based EC_{50} value: 5.2423 ± 1.29 µM (Hill coefficient, n = 0.82)

5. ***Anionophore*** **regeneration study**

5.1. ***Liposomal-based fluorescence study of GSH pretreated proanionophore (6)*** **—** The lucigenin-encapsulated LUVs were prepared as described in the earlier section. To measure the regeneration aptitude, 2940 µL of 20 mM HEPES buffer, pH 7.2, containing 100 mM $NaNO_3$ and 50 µL of EYPC/CHOL-LUV⊃lucigenin were taken in a fluorescence cuvette. Then, it was placed in the fluorescence spectrophotometer at room temperature under a slow stirring condition. The lucigenin fluorescence intensity of the solution was monitored. After 50 s, 10 µL of compound **6** (10 mM GSH pre-incubated for 8h, 15h, and 24h) was added to initiate the anion transport kinetics. At 450 s, the vesicles were lysed by adding 20 µL of 20% Triton X-100 solution, and the fluorescence measurement was continued up to t = 500 s.

5.2. ***HPLC-based study of GSH pretreated proionophore (6)*** **—** HPLC method: In order to confirm the regeneration of active ionophore **3d** from proanionophore **6**, HPLC analysis was performed. Proionophore (10 µM) was incubated in the PBS buffer system with 10 mM GSH in 37 $^{\circ}$C. At time intervals of 8h, 16h, 24 h, 36 h, 48 h, and 72h incubated samples were recorded. Time-dependent study confirms successful release of 3d from proionophore. However, in the absence of GSH, compound 6 integrity remained for 72 hours. Column used: Ascentis® express C18, 2.7 µm HPLC column, flow rate: 0.5 mL/min, mobile phase used: Optimised gradient of PBS buffer/acetonitrile. The gradient used: 0-5 min- 20% buffer:80% CH_3CN, 5-10 min- 10% buffer:90% CH_3CN, 10-14 min- 5% buffer:95% CH_3CN, 14-16 min- 30% buffer:70% CH_3CN.

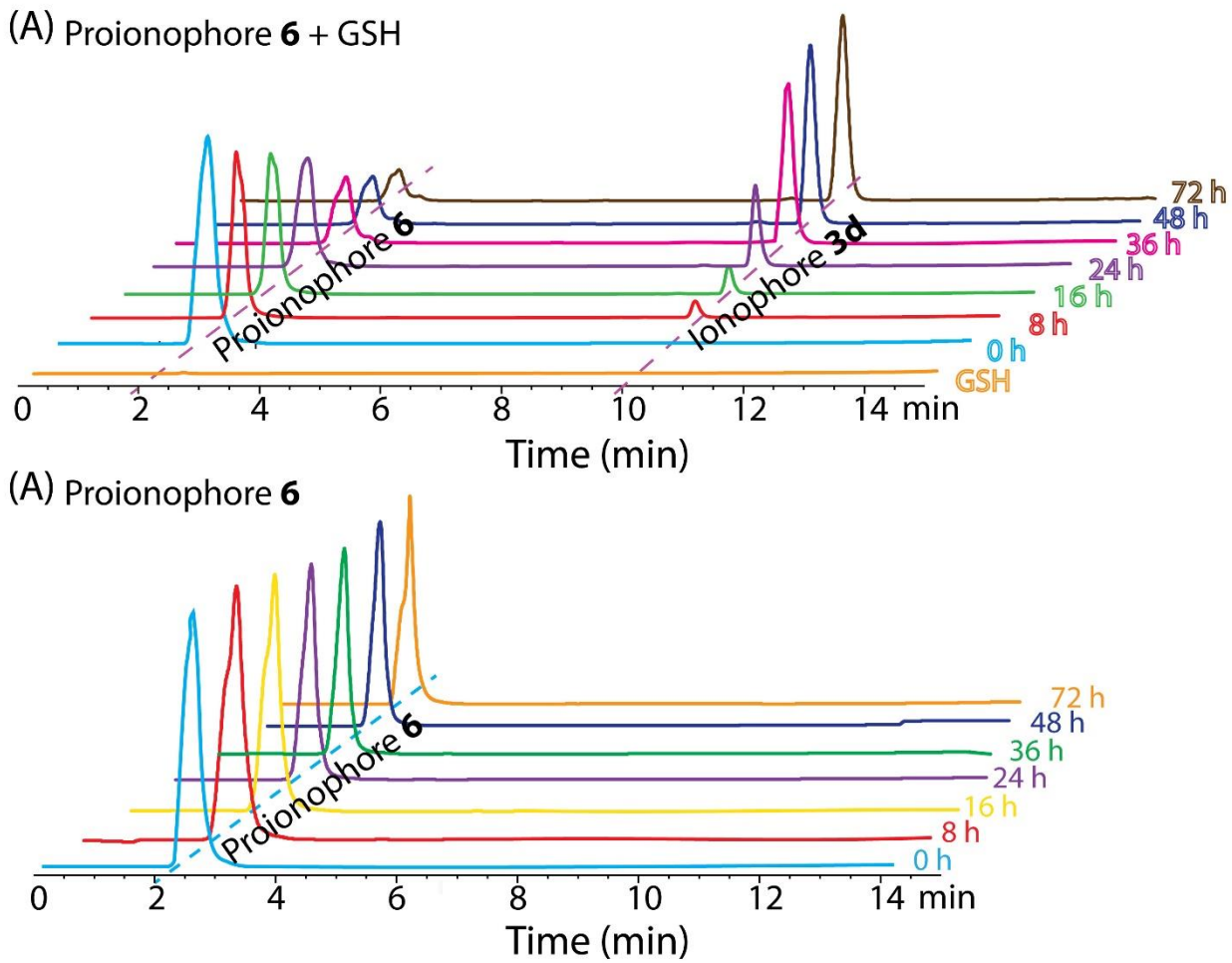


Fig. S4. HPLC-based measurement of active ionophore regeneration upon GSH treatment. Chromatograms of proionophore **6** in the presence (A) and absence of GSH (10 mmol) in PBS (pH = 7.4) at 37 °C. (B).

5.3. Characterization of regeneration of active anionophore by HRMS analysis — The HRMS analysis was performed to confirm the in-situ generation of the active anionophore **3d** from the proanionopore **6** after treatment with GSH. MALDI-TOF analysis of compound **6** showed a peak at 779.357 ($[M/2]^+$). The HRMS of compound **3**, found 559.1052 ($[M+1]^+$) and 1,3-oxathiolan-2-one, found at 103.9556 ($[M]^+$).

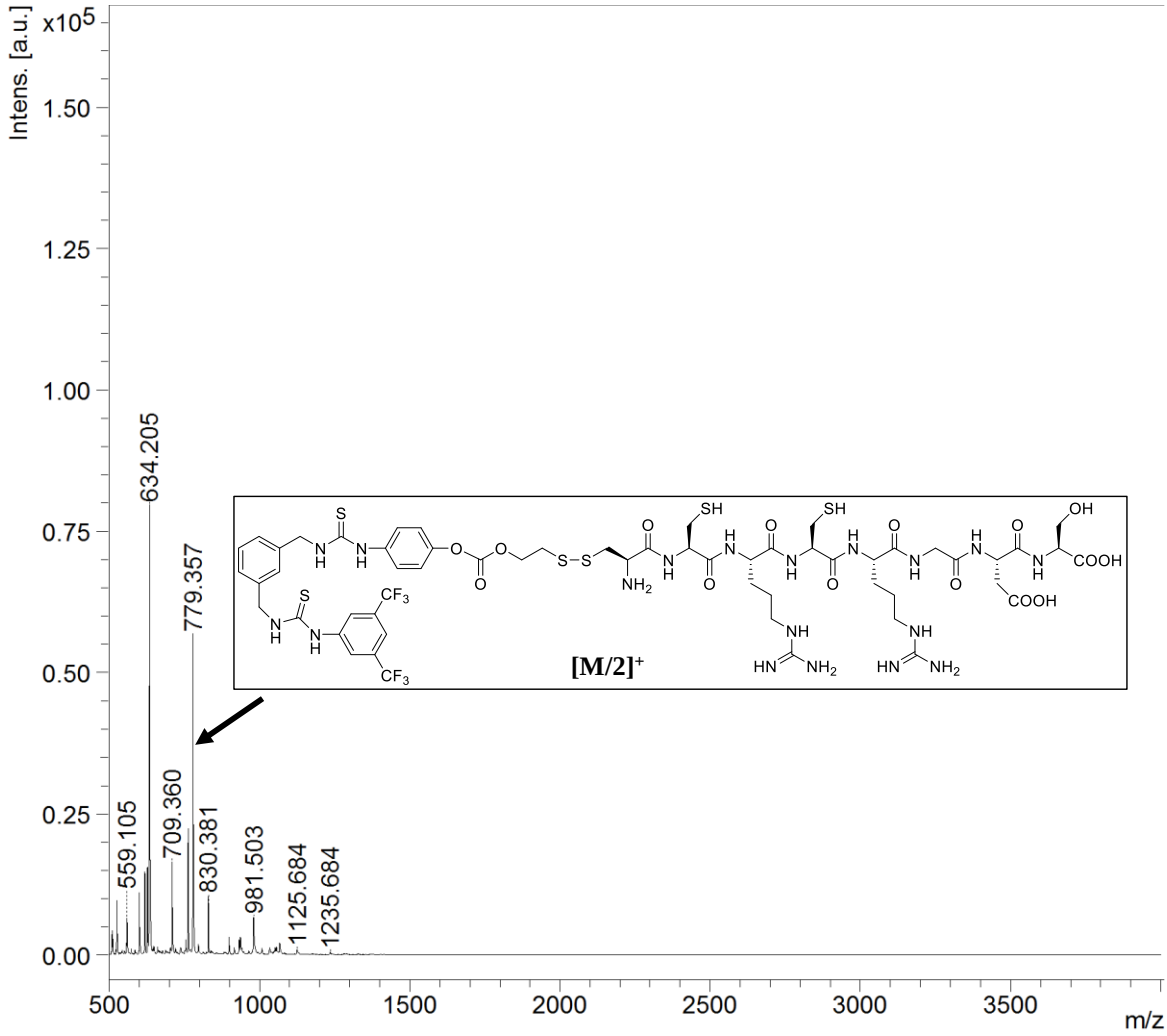


Fig. S5. MALDI-TOF analysis (m/Z above 500) of the proanionopore **6** treated.

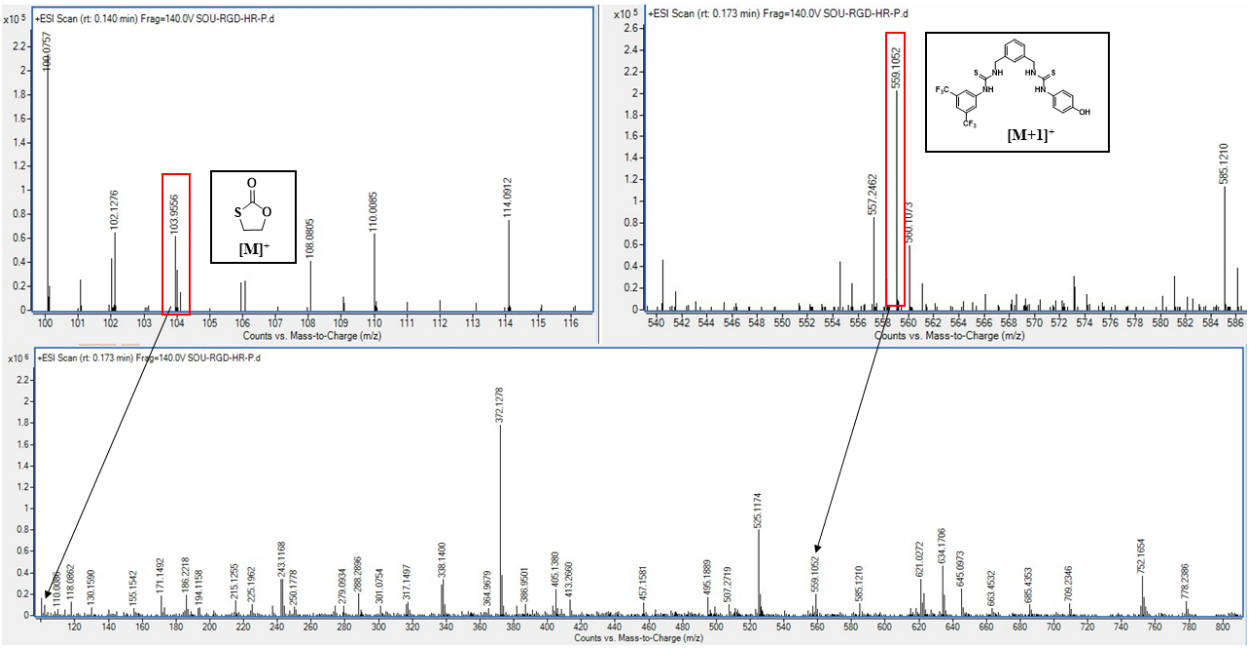


Fig. S6. HRMS analysis of the reaction mixture of proanionopore **6** treated with GSH.

6. ^{1}H NMR titration studies

The stock solutions of **3d** (2 mM) and tetrabutylammonium chloride (TBACl; 200 mM) were prepared in DMSO-d_6 solvent. The ^{1}H NMR spectra were recorded on a Bruker Ascend 600 spectrometer and calibrated with respect to the residual solvent peak in DMSO-d_6. ^{1}H NMR titrations were performed by stepwise addition of TBACl to the solution of the compounds in DMSO-d_6. Upon the addition of TBACl, the changes in the amide N−H chemical shifts were used for calculating the binding constant values. The ^{1}H NMR stacked plots were processed using Mestre Nova 6 software. The association constant was obtained by fitting the data in the 1:1 binding model of the Bindfit v0.5 program.

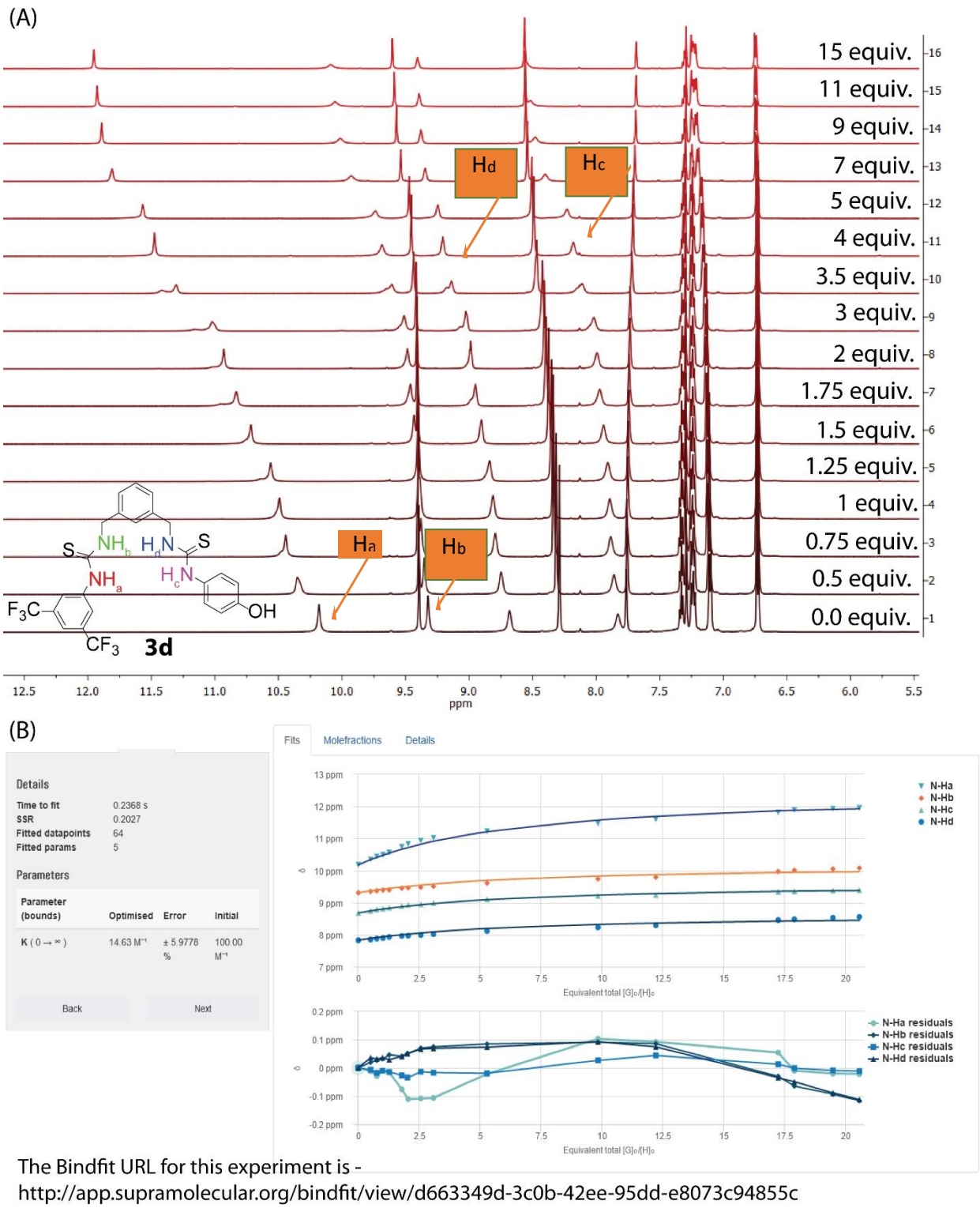


Fig. S7. Partial ^{1}H NMR (600 MHz, rt) titration spectra for compound **3d** (2 mM) with TBACl in DMSO-d_6 solvent. The amounts of added TBACl are shown on the spectra (A). The plot of the concentration of TBACl versus the chemical shift of the ^{1}H signal was fitted using the 1:1 binding model of the Bindfit v0.5 program (B).

7. Mechanistic studies for chloride ion transportation

7.1. ***Cation Selectivity studies across EYPC/CHOL-LUV⊃HPTS*** **—** The cation selectivity studies were performed according to the reported procedure.[4, 5] Briefly, in a clean fluorescence cuvette, 2940 µL of buffer solution (100 mM MCl, 20 mM HEPES, pH 7.2; where M^+ = Li^+, Na^+, K^+, Rb^+, and Cs^+), 50 µL of EYPC/CHOL-LUV⊃HPTS (prepared in 20 mM HEPES buffer containing 100 mM NaCl, pH 7.2) and fluorescence cuvette was placed in a fluorescence instrument equipped with a magnetic stirrer at t = 0 s. The fluorescence emission intensity of the HPTS dye was measured at λ_{em} = 510 nm (where λ_{ex} = 450 nm) for the time course of 0 to 500 s. At t = 50 s, between the intra- and extra-vesicular medium, which leads to the increase in the fluorescence intensity. Triton X-100 (20 µL of 20% solution in water) was added at t = 450 s to destroy all the vesicles.

7.2. ***Cation Selectivity studies across EYPC/CHOL-LUV⊃lucigenin*** **—** The cation selectivity studies were performed according to the reported procedure.(section 4.1.2).[5] Briefly, in a clean fluorescence cuvette, 2940 µL of buffer solution (100 mM MCl, 20 mM HEPES, pH 7.2; where M^+ = Li^+, Na^+, K^+, Rb^+, and Cs^+), 50 µL of above-prepared vesicles solution were taken, and fluorescence cuvette was placed in a fluorescence instrument equipped with a magnetic stirrer at t = 0 s. The fluorescence emission intensity of the lucigenin dye was measured at λ_{em} = 505 nm (where λ_{ex} = 455 nm) for the time course of 0 to 500 s. At t = 50 s, between the intra- and extra-vesicular medium, which leads to the increase in the fluorescence intensity. Triton X-100 (20 µL of 20% solution in water) was added at t = 450 s to destroy all the vesicles.

7.3. ***Anion selectivity studies across EYPC/CHOL-LUV⊃HPTS*** **—** The anion selectivity studies were performed according to the reported procedure.[4] Briefly, 2940 µL of 20 mM HEPES buffer, pH 7.2, containing 100 mM of the respective salt of Na_xA_y (where Na_xA_y = NaCl, NaBr, NaI, $NaNO_3$ and Na_2SO_4) and 50 µL of EYPC/CHOL-LUV⊃HPTS (prepared in 20 mM HEPES buffer containing 100 mM NaCl, pH 7.2) were taken in a fluorescence cuvette. Then, it was placed in the fluorescence spectrophotometer at room temperature under slow stirring conditions. The HPTS fluorescence intensity of the solution was monitored. After 50 s, 10 µL of the respective compound (from DMSO stock) was added to initiate the anion transport kinetics. For the pH gradient study, ΔpH = 0.8 was created by adding 10 µL of 1M NaOH to the extravesicular solution at t = 0 s. At 450 s, the vesicles were lysed by adding 20 µL of 20% Triton X-100 solution, and the fluorescence measurement was continued up to t = 500 s.

7.4. ***Quantitative measurement of transport activity from HPTS assay*** **—** The fluorescence emission intensities of the HPTS dye were normalized, and the intensities appearing at t = 0 and t = 500 s were taken as 0 and 100 units, respectively.[1] 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.

$i.e.\ Transport\ activity,$

$$T_{HPTS} = \frac{F_t - F_0}{F_\infty - F_0} \times 100\% \quad \text{..... Eq (S2)}$$

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 transporter (t = 0 s), and F_∞ = fluorescence intensity after the addition of Triton X-100 solution (i.e., at saturation after complete leakage at t = 500 s).

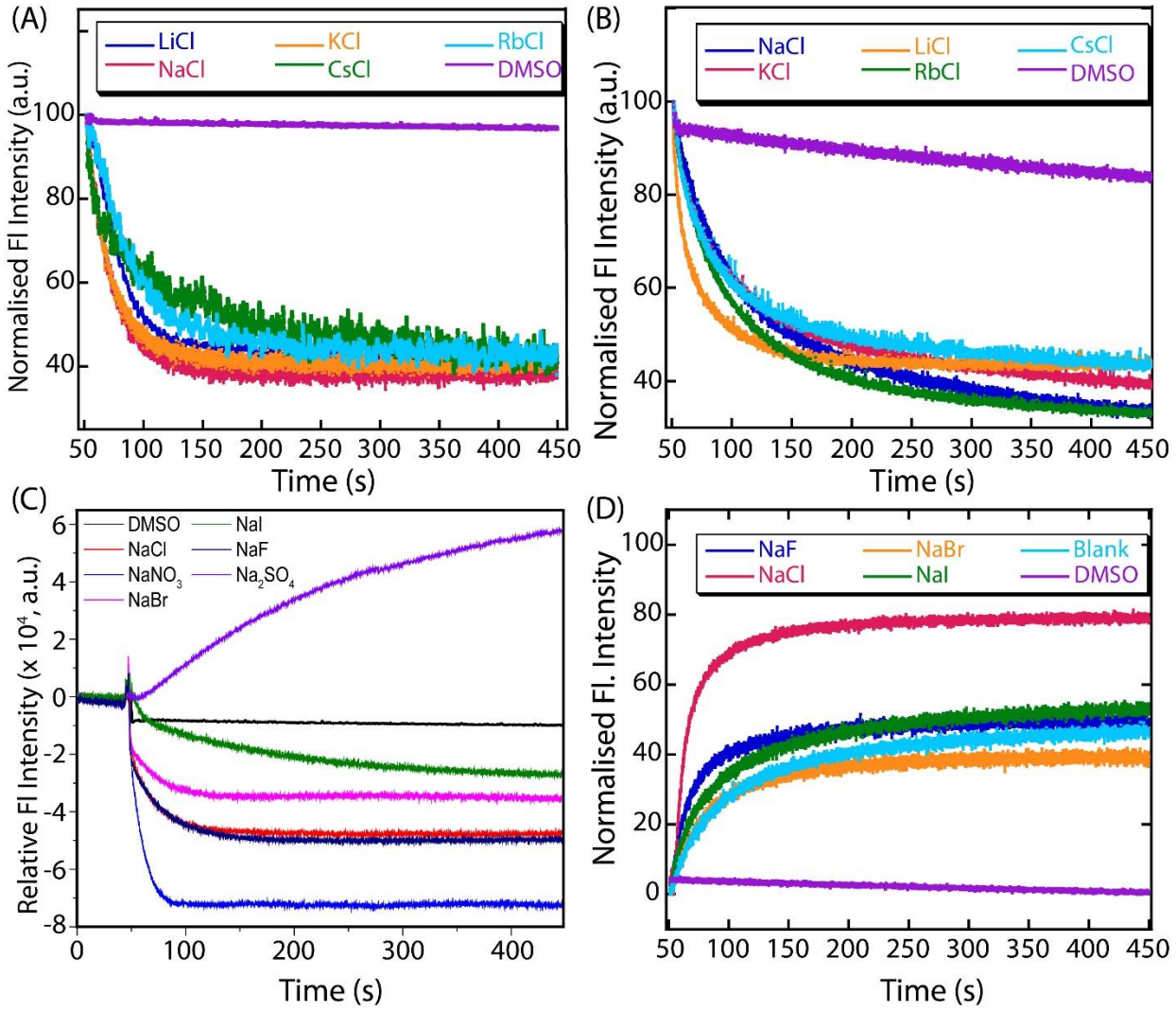


Fig. S8. (A) Cation selectivity study for compound **3d** across EYPC/CHOL-LUVs⊃HPTS where intravesicular solution contains 20 mM HEPES buffers containing 100 mM NaCl, HPTS (1 mM) at pH 7.2 and extravesicular solution contains 20 mM HEPES buffers, 100 mM MCl (where M^+ = Li^+, Na^+, K^+, Cs^+, Rb^+) at pH = 7.2. (B) Cation selectivity study for compound **3d** across EYPC/CHOL-LUVs⊃lucigenin where intravesicular solution contains 20 mM HEPES buffers, 100 mM $NaNO_3$, lucigenin (1 mM) at pH 7.2 and extravesicular solution contains 20 mM HEPES buffers, 100 mM MCl (where M^+ = Li^+, Na^+, K^+, Cs^+, Rb^+) at pH = 7.2. (C) Anion selectivity study compound **3d** using anion gradient assay across EYPC/CHOL-LUVs⊃HPTS where intravesicular solution contains 20 mM HEPES buffers, 100 mM NaCl, HPTS (1 mM) at pH 7.2 and extravesicular solution contains 20 mM HEPES buffers, 100 mM NaX (where X^- = Cl^-, NO_3^-, I^-, Br^-, SO_4^{2-}) at pH = 7.2. (D) Anion selectivity study compound **3d** using dual gradient assay across EYPC/CHOL-LUVs⊃HPTS where intravesicular solution contains 20 mM HEPES buffers,100 mM NaCl, HPTS (1 mM) at pH 7.2 and extravesicular solution contains 20 mM HEPES buffers, 100 mM NaX (where X^- = Cl^-, NO_3^-, I^-, Br^-, SO_4^{2-}) at pH = 8.0.

Note: We performed the anion gradient assay where $NaCl^{in}$/ NaX^{out} (pH 7.2) based on method reported by Gale and co-workers.[6] The outcome of this study showed that compound **3d** in the absence of pH gradient followed higher H^+/X^- influx efficacy over H^+/Cl^- efflux which causes the acidification of intravesicular medium. In the absence of initial pH gradient, a pH gradient is generated by the anion transporter which assist prevailing HX influx or HCl efflux depending on the X^- vs. Cl^- selectivity. The impetus for net H^+ transport is the membrane potential initiating from the permeability variance between X^- and Cl^-. The faster transport of HX over HCl could lead to the acidification of intracellular vesicles.[6] In case of Na_2SO_4, since SO_4^{2-} being highly hydrophilic anion, transporter could not allow it to cross the lipid bilayer causing only H^+/Cl^- efflux. Hence, we saw basification of intravesicular media. (owing to the increased fluorescence intensity due to the formation of $HPTS^-$). We also performed anion selectivity studies with pH gradient (ΔpH = 0.8) mentioned in Fig. S8D, where highest transport rate was observed for Cl^-.[5, 7, 8]

7.5. ***Transport activity in the presence of FCCP —*** The vesicles were prepared by following the same procedure as discussed in the earlier section. The ion transport activity was measured in the absence and presence of FCCP (H^+ selective transporter).[9] First, 2920 µL of 20 mM HEPES buffer, pH 7.2, containing 100 mM NaCl and 50 µL of the EYPC/CHOL-LUV⊃lucigenin was taken in a 3 mL fluorescence cuvette, and the cuvette was placed in the fluorescence spectrophotometer at room temperature under mild stirring condition. After that, the compound (8 µL of the stock solution in DMSO) and 2 µL of FCCP solution in DMSO (1 µM) were added to the solution. The cuvette was then kept inside the fluorescence instrument under stirring conditions for 3 minutes to allow maximum incorporation of the compounds into the lipid bilayers. After that, the lucigenin fluorescence intensity was monitored (t = 0 sec) at 506 nm (λ_{ex} = 455 nm). After 450 sec, the kinetic experiment was terminated by adding 20 µL of 20% Triton-X100 solution (to rupture the vesicular arrangements) into the cuvette, and the fluorescent measurements were continued for another 50 sec (t = 500 sec). The control experiment was performed in the absence of FCCP also.

7.6. ***Preferential ion transport activity in the presence of valinomycin (valinomycin assay) —*** For the valinomycin assay, the EYPC/CHOLLUV⊃lucigenin was prepared by following a similar procedure as mentioned in the earlier section.[9] The extracellular buffer was replaced with 100 mM KCl with 100 mM $NaNO_3$. Here at 50 s, the performed as solution, the respective compound (from DMSO stock) and /or 2 µL valinomycin (12.0 pM) of 10 µL of was added to initiate the anion

transport kinetics. The fluorescence emission intensity of the lucigenin dye was normalized at t = 50, and t = 500 s and transferred as 0 and 100 units, respectively.

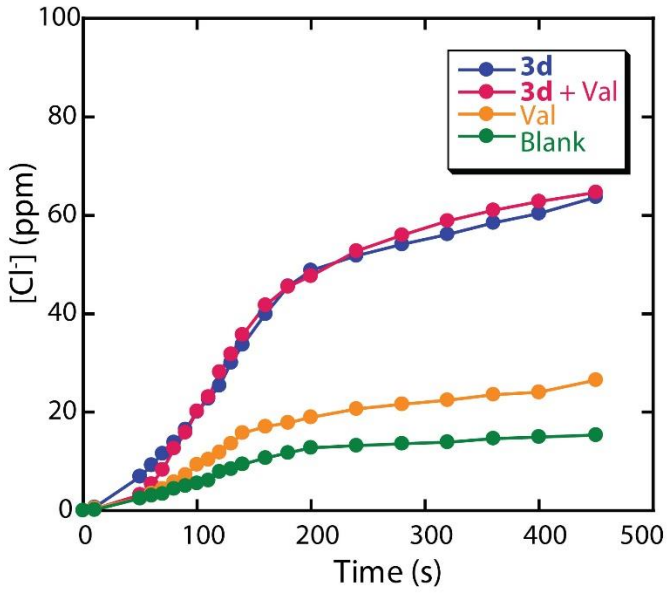


Fig. S9. Chloride ion efflux activities of compound **3d** in the presence and absence of valinomycin. Val = valinomycin.

7.7. ***Evidence for the carrier mechanism*** — Lucigenin assay using 1,2-dipalmitoylphosphatidylcholine (DPPC) was employed to determine the transport mechanism of the compounds.[9] DPPC-LUV⊃lucigenin were prepared in 20 mM HEPES buffer pH 7.2, containing 100 mM $NaNO_3$. For the preparation of DPPC-LUV⊃lucigenin first 50 µL of DPPC (100 mg/mL stock in de-acidified $CHCl_3$) was taken in a clean and dry glass vial, and the organic solvent was removed under reduced pressure (for 5-6 h) at room temperature. The dry, thin film was then hydrated with 600 µL of 20 mM HEPES buffer, pH 7.2, containing 1 mM lucigenin and 100 mM $NaNO_3$. The solution was then sonicated for 30 min at 50 °C and was vortexed occasionally for 15-20 minutes. After that, the solution was subjected to a freeze-thaw cycle 12-13 times and again was sonicated 10 times (40-sec sonication followed by 20-sec incubations in ice water). The LUVs were prepared by extrusion using Avanti Mini-Extruder (Avanti Polar Lipids, Alabaster, AL) through 200 nm pore-size polycarbonate membranes according to the manufacturer's protocol. The unencapsulated dye was removed using a gel filtration (Sephadex G-50) column with 20 mM HEPES buffer, pH 7.2, containing 100 mM $NaNO_3$. The final volume of the collected vesicle solution was adjusted to 500 µL with 20 mM HEPES buffer pH 7.2 containing 100 mM $NaNO_3$. The final lipid concentration was 13.62 mM (assuming 100 % lipid

regeneration). For the transport activity assay, the first 2920 µL of 20 mM HEPES buffer, pH 7.2, containing 100 mM $NaNO_3$, 50 µL of the DPPC-LUV⊃lucigenin (concentration of stock solution = 13.62 mM), and 20 µL of 5M NaCl was taken in a 3 mL fluorescence cuvette, and the cuvette was placed in the fluorescence spectrophotometer under slow stirring condition. After that, compounds (10 µL from a 5 µM stock solution in DMSO) were added to the solution to achieve a concentration ratio of 1: 25,000 for compound and lipids. The lucigenin fluorescence-based kinetic measurements were performed as mentioned above.

7.8. ***Transport Activity across DPPC-LUV⊃lucigenin (DPPC Assay)*** **—** DPPC assay was performed using a Fluoromax-4 spectrofluorometer (Horiba Scientific, Singapore) connected with a refrigerated system for temperature control (where the temperature was regulated using a temperature controller).[10] In this assay, 2920 µL of 20 mM HEPES buffer, pH 7.2 containing 100 mM $NaNO_3$, and 50 µL of the DPPC-LUV⊃lucigenin were taken in a 3 mL fluorescence cuvette. 10 µL of DMSO stock solution of the compounds 1e and 1f was added to the cuvette (to make the anionophore and lipid ratio of 1: 25,000).15 The kinetic experiment was started (at t = 0 s), and lucigenin fluorescence emission was monitored, as mentioned above. The cuvette was then kept under stirring conditions, and the chamber temperature was set to 25 ºC. After 50 sec, NaCl (20 µL, 5 M) was added to initiate the Cl^- influx kinetics. Finally, to terminate the kinetic experiment, the vesicles were lysed by adding 20% Triton X-100 (20 µL) in the cuvette at t = 450 sec, and fluorescent measurements were continued for another 50 sec (i.e., up to t = 500 sec).

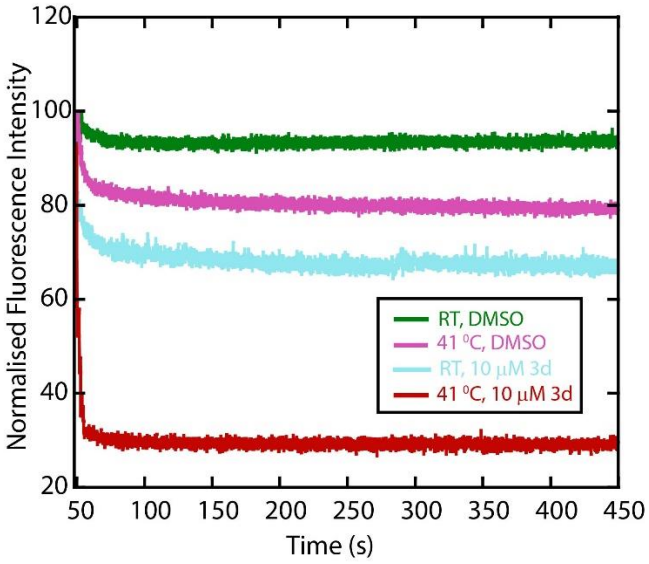


Fig. S10. Temperature-dependent lucigenin assay to demonstrate the carrier-mechanistic pathway of Cl^- transport activity of compound **3d** across DPPC-LUV⊃lucigenin at 25 °C and 41 °C.

7.9. ***U-tube experiment*** **—** The Classical U-tube experiment was performed according to the reported procedure to confirm the mechanistic pathway for the Cl^- transport by the compounds. The lipid bilayer was mimicked by using chloroform (12 mL) as the organic layer. The compound (2 mM) in chloroform was placed at the bottom of the U-tube with a mild stirring condition. The left arm of the tube was filled with 0.1 M aqueous HCl solution (10 mL), and the right one was filled with 0.1 M aqueous $NaNO_3$ solution (10 mL). The Cl^- concentration of the receiver end was monitored using a chloride-ISE. In the meantime, the pH of the receiver end was monitored using a pH meter. H^+/Cl^- cotransport by the compound was observed by monitoring the right arm of the tube using both a pH meter and ISE.

7.10. ***Preparation of EYPC−LUV*⊃carboxyfluorescein** **—** A thin lipid film was prepared by evaporating a solution of 154 µL EYPC (50 mg/mL stock in chloroform), and 39 µL cholesterol (25 mg/mL stock in chloroform) in vacuo for 4 h.[11] After that lipid film was hydrated with 800 μL buffer (10 mM HEPES, 10 mM NaCl, 50 mM carboxyfluorescein (CF), pH 7.2) for 1 h with occasional vortexing of 4−5 times and then subjected to freeze-thaw cycle (≥ 15 times). The vesicle solution was extruded through a polycarbonate membrane with 200 nm pores 19 times (has to be an odd number) to give vesicles with a mean diameter of ~ 200 nm. The extracellular dye was removed with size exclusion chromatography (Sephadex G-50) with 10 mM HEPES buffer (100 mM NaCl, pH 7.2. Final) Final concentration: ~25 mM EYPC-CHOL lipid; intravesicular solution: 10 mM HEPES, 10 mM NaCl, 50 mM CF, pH 7.2; extravesicular solution: 10 mM HEPES, 100 mM NaCl, pH 7.2.

7.11. ***Carboxyfluorescein leakage assay*** **—** In a clean and dry fluorescence cuvette, 50 µL of the above lipid solution and 2940 µL of 10 mM HEPES buffer 100 mM NaCl, pH 7.2 was taken and kept in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at t = 0 s). The CF fluorescence emission intensity time course, F_t, was observed at λ_{em} = 517 nm (λ_{ex} = 492 nm). Compound **3d** was added at t = 50 s, and at t = 450 s, 20 µL of 20% Triton X-100 was added to lyse those vesicles for 100% chloride influx. This study confirmed that the integrity of the bilayer membranes is intact in the presence of compound **3d**.

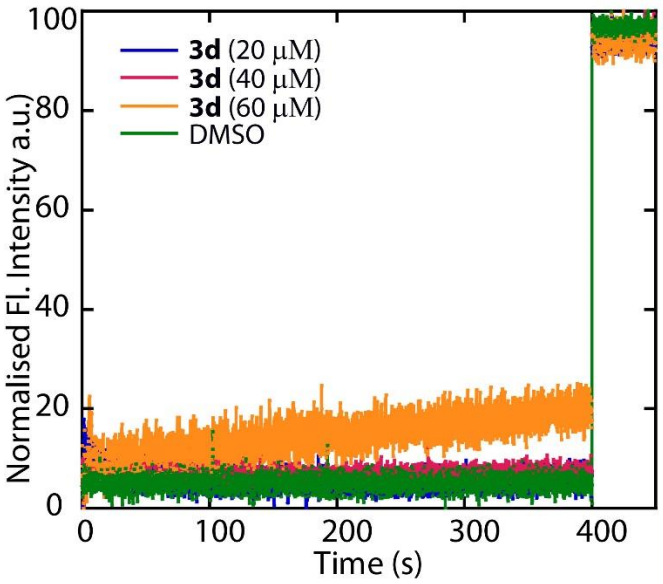


Fig. S11. Carboxy fluorescein leakage assay of compound **3d** across EYPC-CHOL-LUVs.

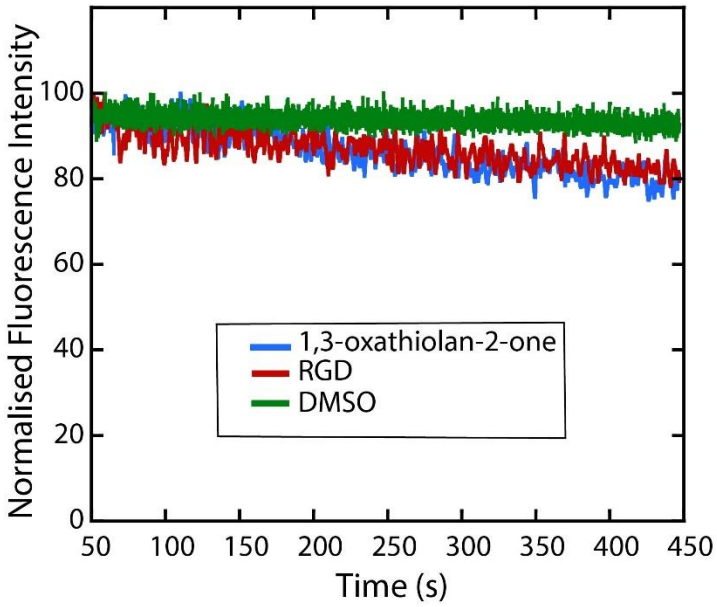


Fig. S12. The Cl^- transport activity of control compounds.

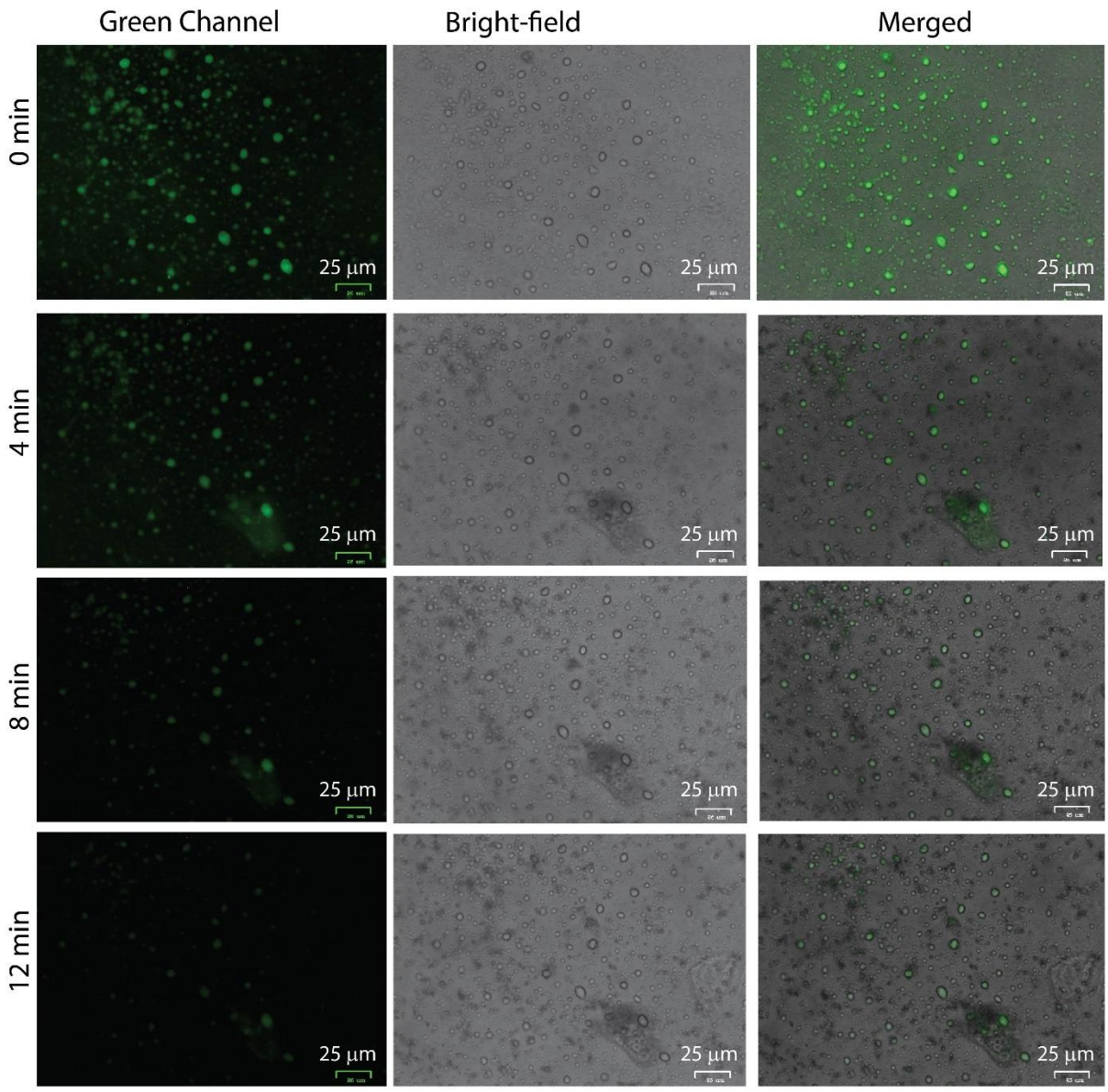


Fig. S13. The time-dependent confocal microscopic images of the giant unilamellar vesicles (GUVs) after the addition of **3d**.

8. Cellular studies

8.1. ***Cell culture*** **—** HEK293T human kidney epithelial, A375 human melanoma cell lines, and HeLa cervical cancer, were cultured in DMEM medium. The culture medium for all the cell lines was supplemented with 10% FBS (Gibco, USA), antibiotics (Gibco, USA), and L-glutamine (Gibco, USA). They were maintained routinely at 37°C, in a humidified atmosphere with 5% CO_2 in an incubator (Eppendorf) in the cell culture laboratory. For each experiment, cells were first trypsinized, counted with the help of a cell counter (Countess 3, Invitrogen, USA), and then seeded in multi-well cell culture plates as per the experimental requirement.

8.2. ***MTT-cell viability assay in DMEM cell culture media*** **—** MTT cell viability assays were performed to assess the effect of the reported compound on the viability of the normal HEK293T or the cancer cell A375. Briefly, $1x10^4$ numbers of HEK293T or A375 cells were seeded in 96 well cell culture plates with 100 µL complete DMEM media and incubated overnight to allow the cells to attach. After attaining confluency, cells were treated with different concentrations of compounds 3d or 6 in different conditions, such as 10% FBS, and 1% FBS. After the treatment, MTT solution (10 µL of 5 mg MTT/mL of PBS) was added to each plate and incubated for 4 hours to allow the formation of formazan crystals. Next, formazan crystals were dissolved by using acidified IPA and incubated for 30 minutes at 37°C The absorbance was measured using a multimode microplate reader (EPOCH, BioTek, Agilent, USA) at 570 nm. All experiments were carried out at least three times, and the relative cell viability (%) was expressed as a percentage of untreated/control cells.

8.3. MTT-cell viability assay in HBSS buffer — MTT assays were repeated in the A375 cell line using HBSS (Hank's balanced salt solution) buffer in the presence and absence of Cl^-. Hank's balanced salt solution with Cl^- has the following composition: 136.9 mM NaCl, 5.5 mM KCl, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 0.81 mM $MgSO_4$, 1.25 mM $CaCl_2$, 5.5 mM D-glucose, 4.2 mM $NaHCO_3$ and 10 mM HEPES (pH 7.4) Hank's balanced salt solution without Cl^- was prepared using 136.9 mM Na-gluconate, 5.5 mM K-gluconate, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 0.81 mM $MgSO_4$, 1.25 mM Ca-gluconate, 5.5 mM D-glucose, 4.2 mM $NaHCO_3$ and 10 mM HEPES (pH 7.4). Briefly, $1x10^4$ numbers of A375 cells were seeded in 96 well cell culture plates with 100 µL complete DMEM media, and after overnight incubation, the media was replaced with HBSS buffer (with Cl^- and without Cl^-) containing 10% FBS for drug treatment. Cells in HBSS buffers were exposed to different doses of compound 3d and incubated for 24 h. After the treatment, MTT solution was added to each plate and incubated for 4 hours. Next, formazan crystals were dissolved by using acidified IPA, and the absorbance was measured using a multimode microplate reader (BioTek, Agilent, USA) at 570 nm. All experiments were carried out at least three times, and the relative cell viability (%) was expressed as a percentage of untreated/control cells.

Statistical Analysis

For all the biological experiments, at least 3 different experiments were performed, and the data are presented as mean ± SD.

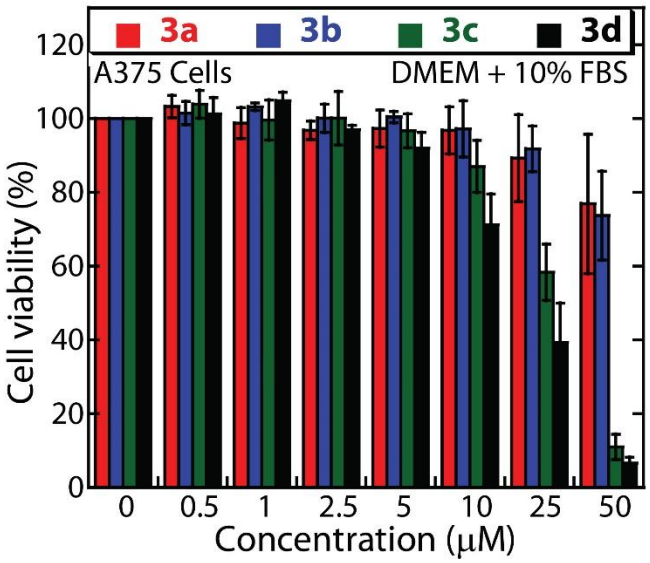


Fig. S14. Viability of A375 cells treated (24 h) with selected compounds.

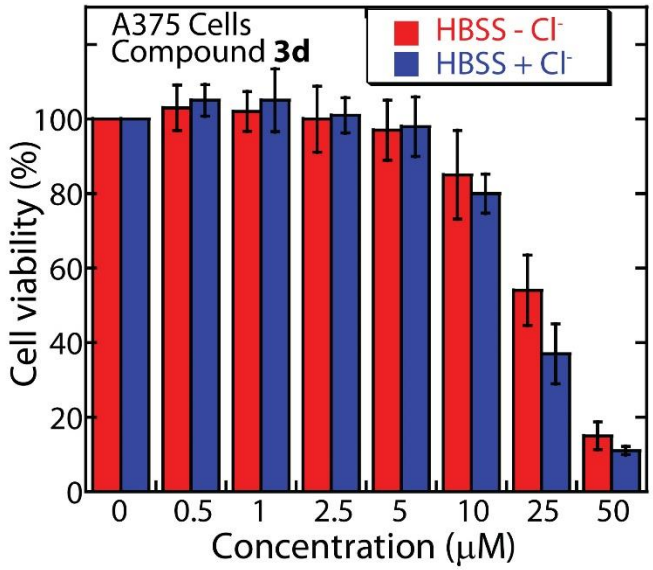


Fig. S15. Viability of A375 cells in HBSS buffer with or without Cl^- at different concentrations of compound **3d**.

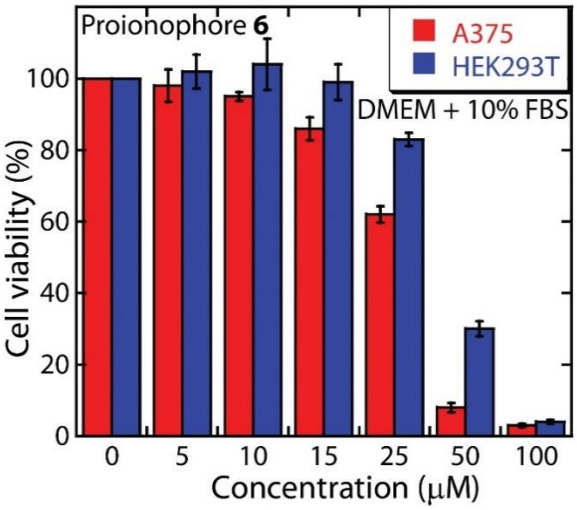


Fig. S16. Viability of A375 and HEK293T cells treated (24 h) with different concentrations of proionophore **6** in the presence of 10% FBS.

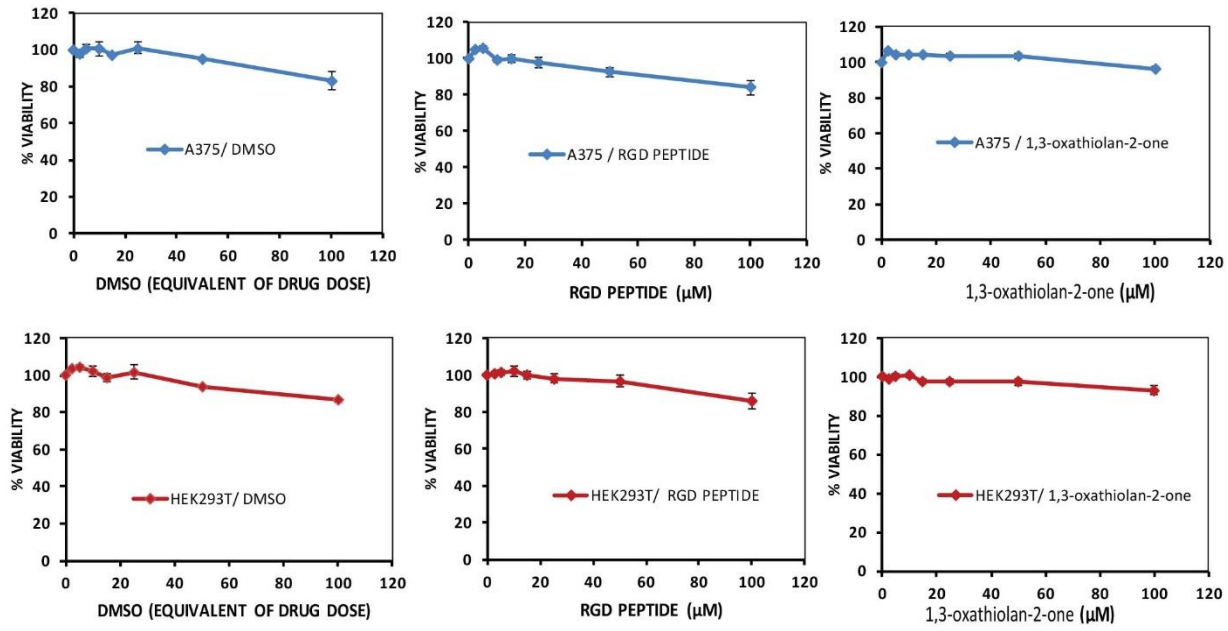


Fig. S17. Viability of A375 and HEK293T cells treated (24 h) with different concentration of DMSO, RGD-peptide and 1,3-oxathiolan-2-one in the presence of 10% FBS.

Note: The compound reported by Gale and coworkers are bisurea moieties (Chem. Sci., 2013, 4, 103-117), whereas we reported phenylene-based bisthiourea compounds with a free phenolic OH group. Gale and coworkers reported that molecules follow Cl^-/NO_3^- antiport as well as H^+/Cl^- symport pathway, but the carrier molecule **3d** has specifically H^+/Cl^- transport activity. Also, our anionophore was attached to RGD peptide via carbonate and S-S linker, making it more interesting because the proionophore **6** showed 5.4-fold less toxicity towards normal cells over cancer cell line. The RGD-peptide assists in selectively delivering the ion carriers to the cancer cells over normal cells. We presume there is a significant difference in the structure and activity of the ionophores, as reported by us and Gale (Chem. Sci., 2013, 4, 103-117).

9. HPLC-based analysis of anionophore (3d) regeneration from control proanionophore (7)

The HPLC analysis was performed to confirm the regeneration of active ionophore **3d** from the control proanionophore **7**. The control proanionophore **7** (10 µM) was incubated in the PBS buffer system with 10 mM GSH at 37 $^{\circ}$C. At the time interval of 12 h incubated samples were recorded. Time-dependent studies confirm the successful release of **3d** from the control proanionophore **7**. (Detector: UV light used of 250 nm). HPLC analysis was performed using Ascentis® express C18, 2.7 µm HPLC column. The optimized gradient mobile phase of PBS buffer/acetonitrile was used with a 0.5 mL/min flow rate. This study confirmed the successful release of **3d** from the control proionophore **7**. (UV light used: 250 nm). The gradient used: 0-5 min- 40% buffer:60% CH_3CN; 5-10 min- 20% buffer:80% CH_3CN; 10-12 min- 5% buffer: 95% CH_3CN; 12-15 min- 50% buffer: 50% CH_3CN.

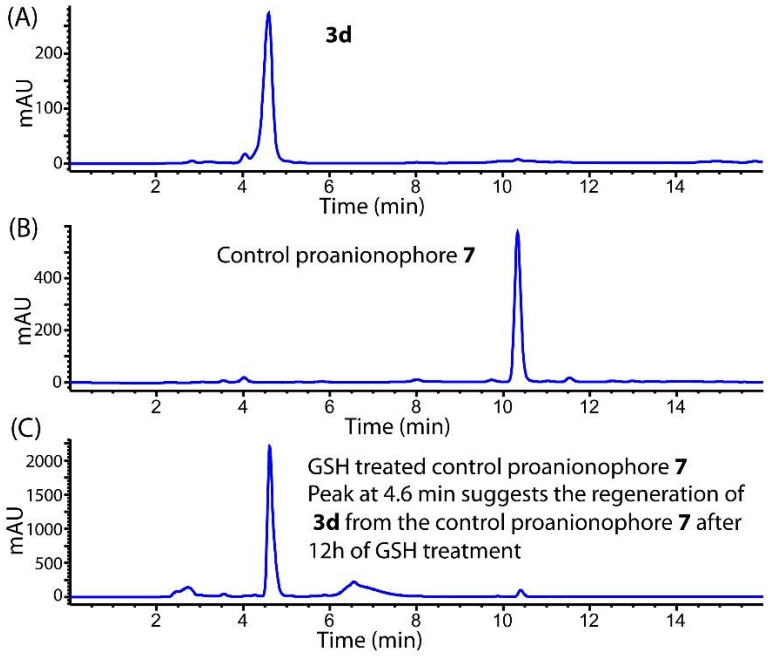


Fig. S18. HPLC-based regeneration assay for control proanionophore **7**. HPLC traces of only anionophore **3d** (A), only control proanionophore **7** (B), and GSH-treated control proanionophore **7** for 12 h (C).

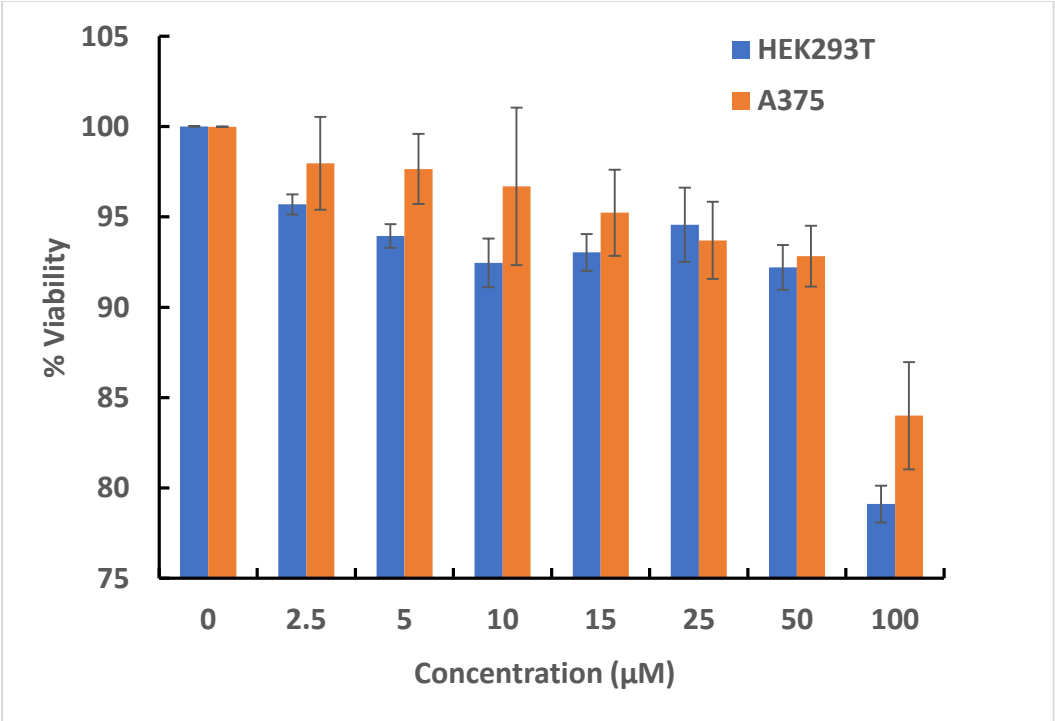


Fig. S19. Viability of HEK293T and A375 cells treated (24 h) at different concentrations of control proanionophore **7.**

9. NMR Spectra of the synthesized compounds

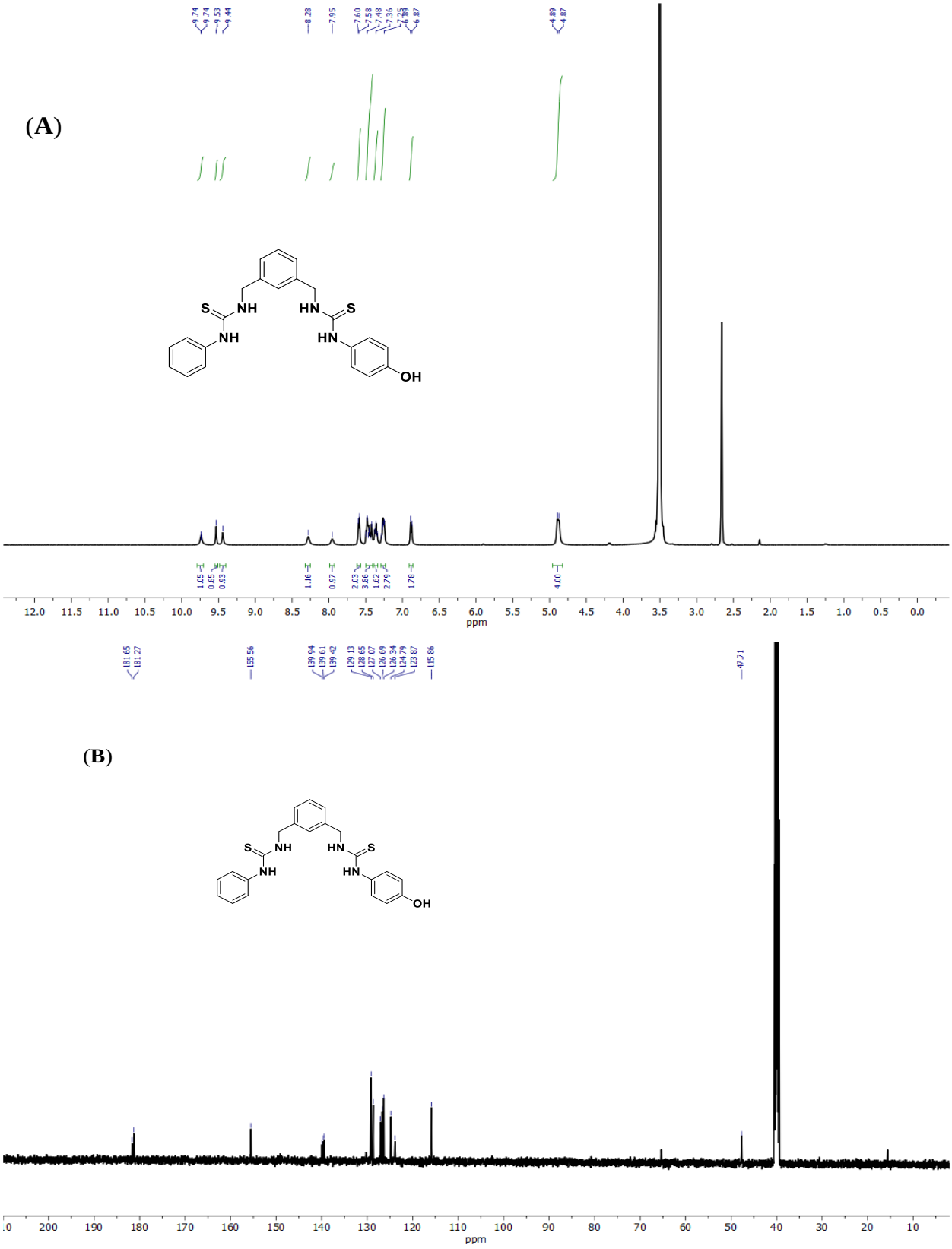


Fig. S18. ^{1}H (A) and ^{13}C (B) NMR of **3a** in DMSO-d_6.

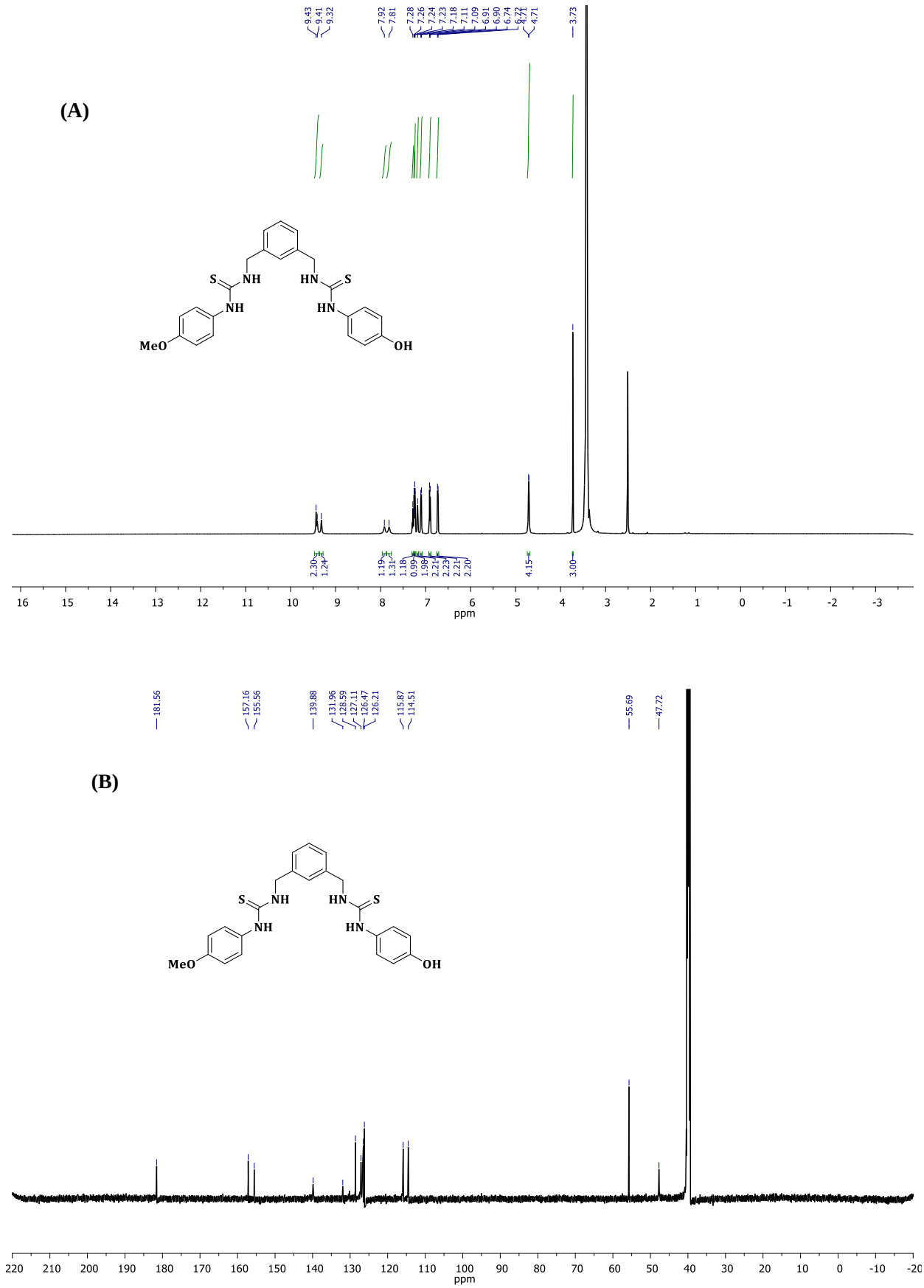


Fig. S19. ^{1}H (A) and ^{13}C (B) NMR of **3b** in DMSO-d_6.

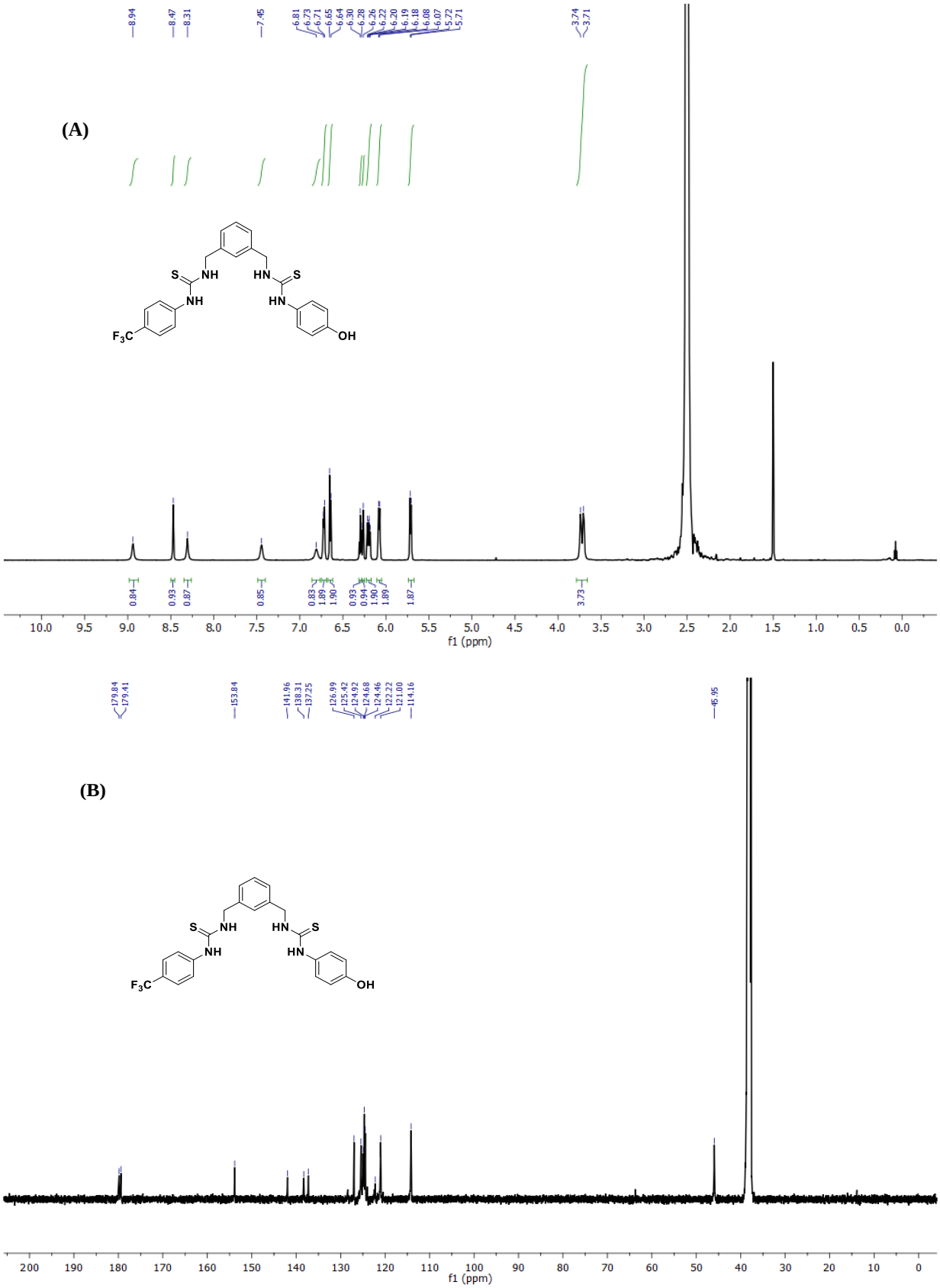


Fig. S20. ^{1}H (A) and ^{13}C (B) NMR of **3c** in DMSO-d_6

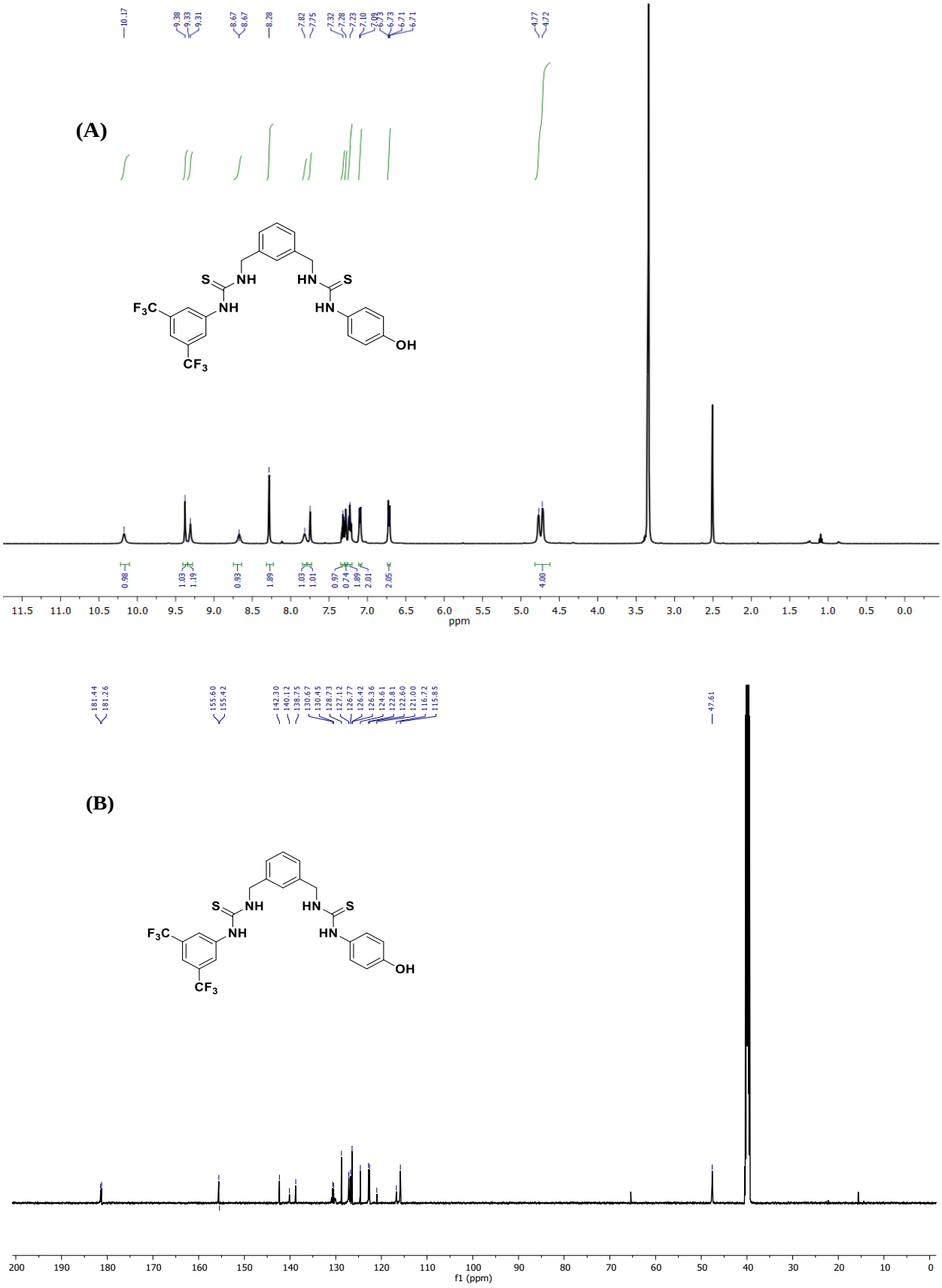


Fig. S21. ^{1}H (A) and ^{13}C (B) NMR of **3d** in DMSO-d_6.

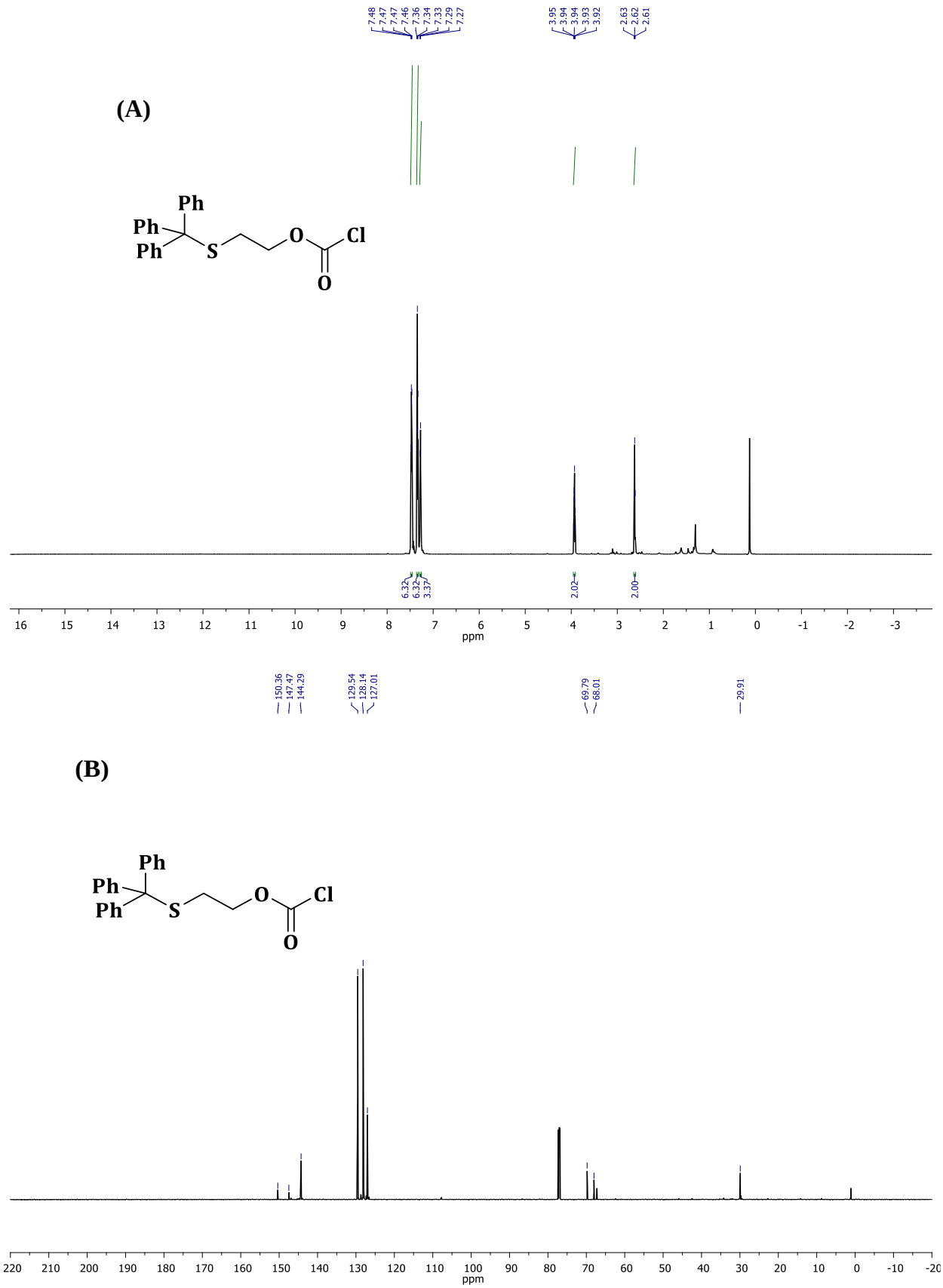


Fig. S22. ^{1}H (A) and ^{13}C (B) NMR of **4** in DMSO-d_6.

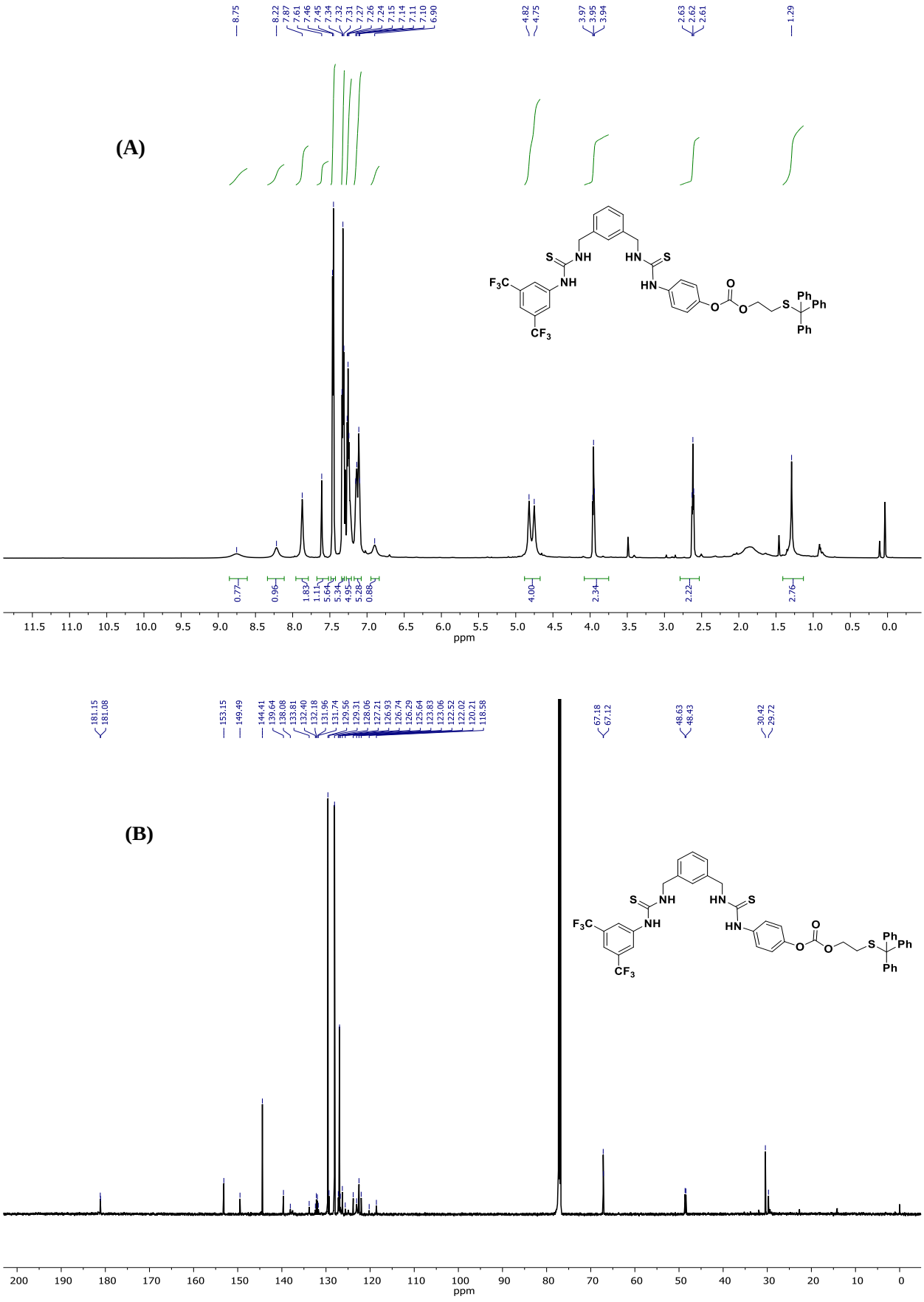


Fig. S23. ^{1}H (A) and ^{13}C (B) NMR of **5** in DMSO-d_6.

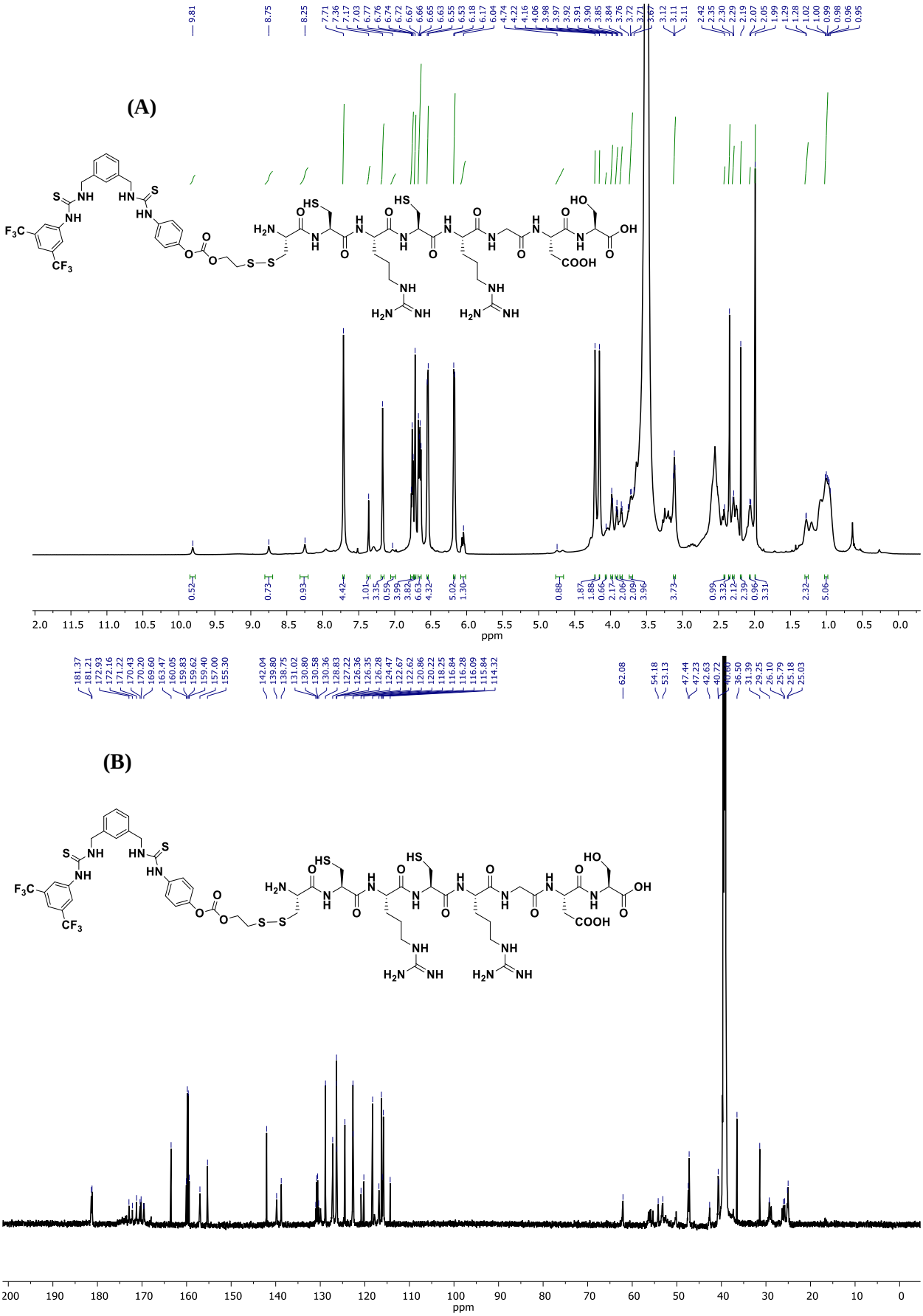


Fig. S24. ^{1}H (A) and ^{13}C (B) NMR of **6** in DMSO-d_6.

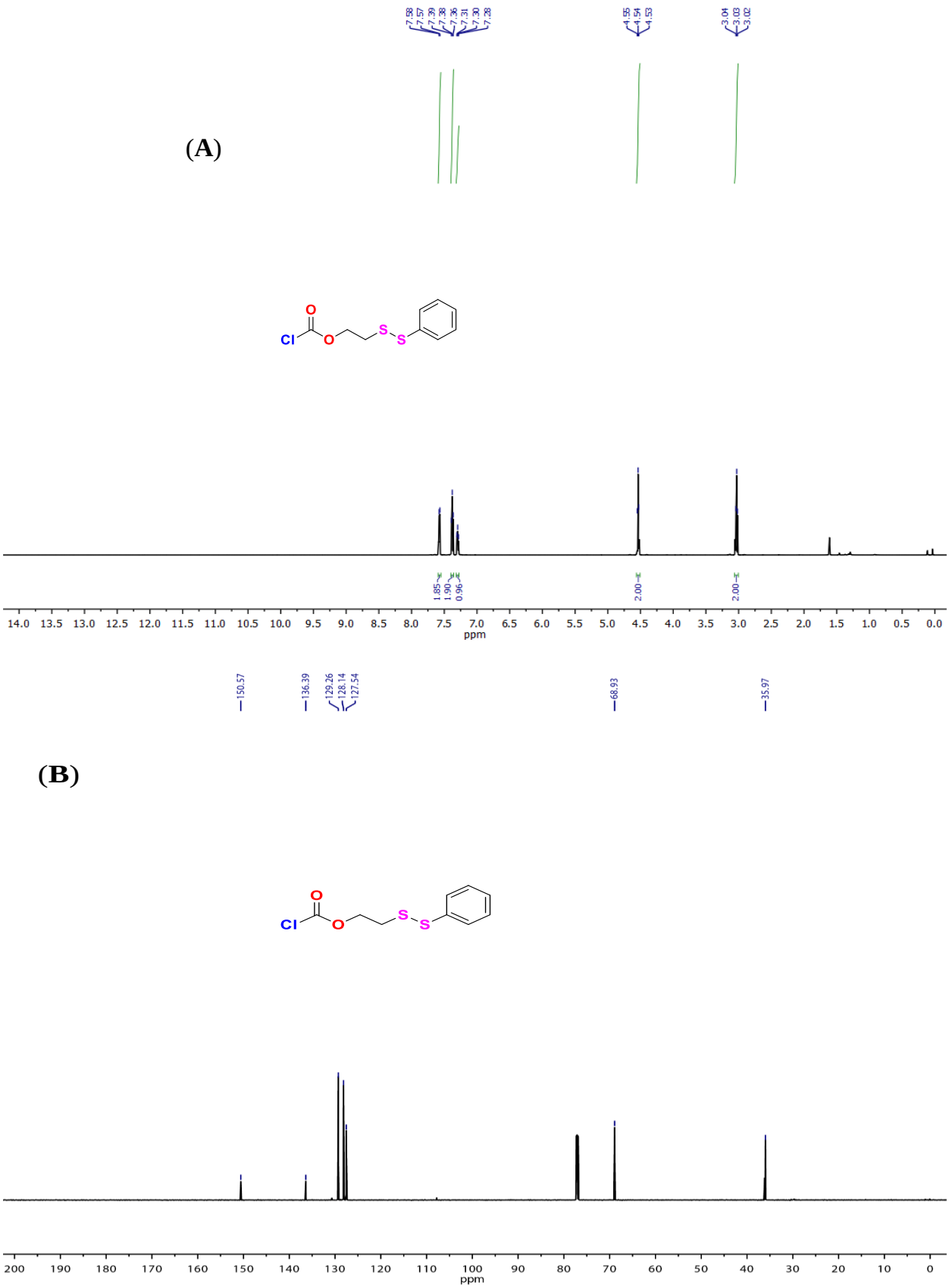


Fig. S25. ^{1}H NMR (A) and ^{13}C NMR (B) of 2-(phenyldisulfaneyl)ethylcarbonochloridate in $CDCl_3$ solvent.

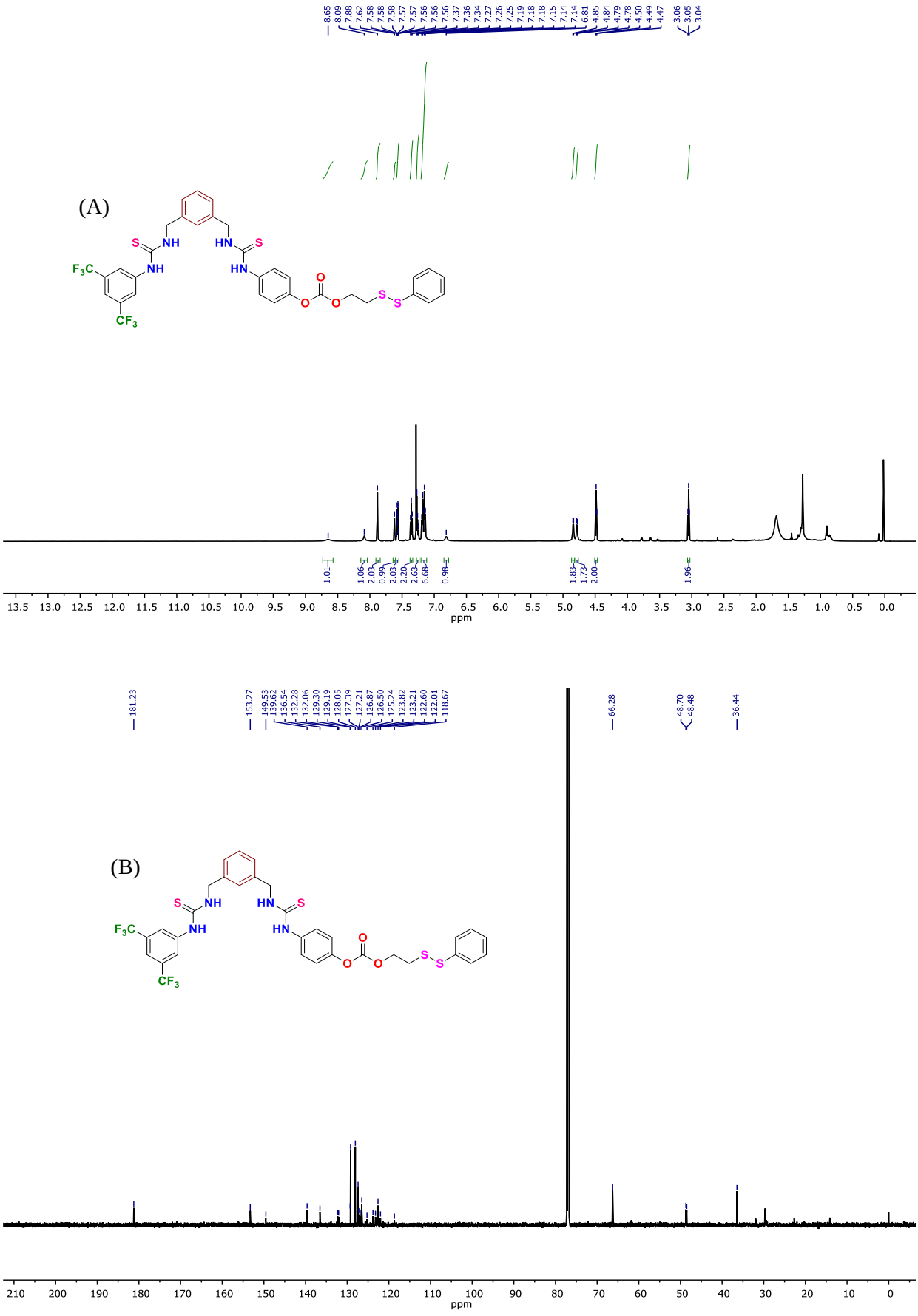


Fig. S26. ^{1}H NMR (A) and ^{13}C NMR (B) of compound **7** in $CDCl_3$ solvent.

9. References

1. S. Das, O. Biswas, N. Akhtar, A. Patel and D. Manna, *Org. Biomol. Chem.*, 2020, **18**, 9246-9252.

2. L. Rocard, A. Berezin, F. De Leo and D. Bonifazi, *Angew. Chem. Int. Ed.*, 2015, **54**, 15739-15743.

3. N. Akhtar, N. Pradhan, A. Saha, V. Kumar, O. Biswas, S. Dey, M. Shah, S. Kumar and D. Manna, *Chem. commun.*, 2019, **55**, 8482-8485.

4. J. A. Malla, A. Upadhyay, P. Ghosh, D. Mondal, A. Mondal, S. Sharma and P. Talukdar, *Org. Lett.*, 2022, **24**, 4124-4128.

5. N. J. Roy, S. N. Save, V. K. Sharma, B. Abraham, A. Kuttanamkuzhi, S. Sharma, M. Lahiri and P. Talukdar, *Chem-Eur J*, 2023, **29**, e202301412.

6. X. Wu and P. A. Gale, *Chem. Commun.*, 2021, **57**, 3979-3982.

7. S. V. Shinde and P. Talukdar, *Chem. Commun.*, 2018, **54**, 10351-10354.

8. S. V. Shinde and P. Talukdar, *Angew. Chem. Int. Edit.*, 2017, **56**, 4238-4242.

9. N. Akhtar, N. Pradhan, G. K. Barik, S. Chatterjee, S. Ghosh, A. Saha, P. Satpati, A. Bhattacharyya, M. K. Santra and D. Manna, *ACS Appl. Mater. Interf.*, 2020, **12**, 25521-25533.

10. O. Biswas, N. Akhtar, Y. Vashi, A. Saha, V. Kumar, S. Pal, S. Kumar and D. Manna, *ACS Appl. Bio Mater.*, 2020, **3**, 935-944.

11. J. A. Malla, A. Upadhyay, P. Ghosh, D. Mondal, A. Mondal, S. Sharma and P. Talukdar, *Org. Lett.*, 2022, **24**, 4124-4128.