Supporting Information

# Molecular clips with spatially proximal urea residues for efficient transmembrane co-transport of $H^+/Cl^-$ ions

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## 1. Materials and Methods

### 1.1 *General Information*

All the chemicals and solvents used for synthesis and characterization were procured from commercial sources like Sigma-Aldrich, Spectrochem, TCI Chemicals, and Merck and used without further purification. Egg yolk phosphatidylcholine (EYPC), 1,2-dipalmitoyl-*sn*-glycerol-3-phosphocholine (DPPC) lipids, cholesterol, mini extruder, and polycarbonate membranes used for the preparation of LUVs were purchased from Avanti Polar Lipids. For size-exclusion chromatography, a column packed with Sephadex G-50 was used. Autoclaved Mili-Q water was used for the preparation of the buffer solutions.

### 1.2 *Methods*

**High-resolution mass spectrometry (HR-MS)−** The HR-MS data of the compound was recorded using Bruker MicrOTOF-Q-II mass spectrophotometer in electrospray ionization (ESI) mode.

**Nuclear Magnetic Resonance (NMR)−** $^{1}H$ and $^{13}C$ Nuclear Magnetic Resonance (NMR) studies were performed on Bruker Ultra Shield (400 MHz and 500 MHz) spectrometers with TMS (tetramethylsilane) as the internal standard.

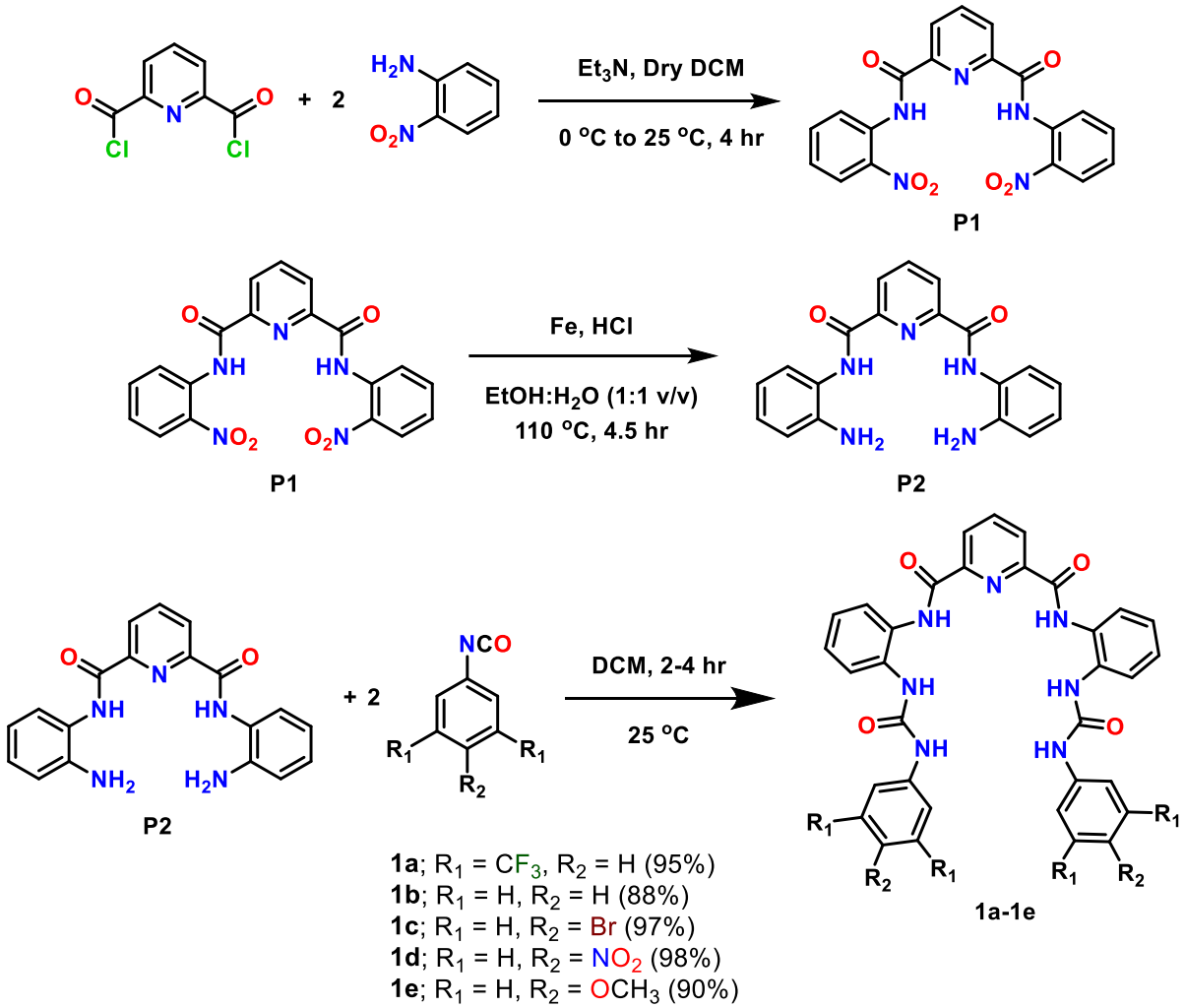
**Single crystal X-ray diffraction (SC-XRD)−** Single Crystal X-ray Diffraction data were collected on a Bruker D8 Venture diffractometer equipped with a Photon-III detector using S4 monochromated Mo Kα radiation (λ = 0.71073 Å) at 140 K using an Oxford cryo-stream low-temperature device. Unit cell measurement, data integration, scaling, and absorption corrections for the crystal were done with Bruker APEX II software. Data reduction was carried out with Bruker SAINT suite. Absorption correction was performed by the multi-scan method implemented in SADABS. All the crystal structures were solved by direct methods using SIR 2014. The crystal structure refinements were done in the program package OLEX2.[1]

**Dynamic Light Scattering (DLS)−** The average diameter of the LUVs was determined by Dynamic Light Scattering (DLS) using a Delsa Nano (Beckman Coulter) instrument with the CONTIN algorithm.

**Fluorescence Studies−** Fluorescence spectra were recorded on Jobin Yvon Horiba's Fluorolog-3-21 instrument with an inbuild magnetic stirrer and temperature controller.

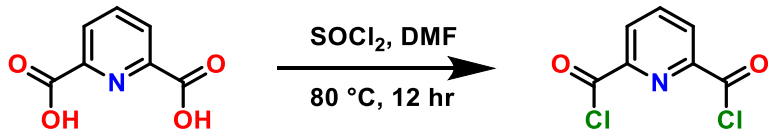
**DFT Calculations−** The molecular structure optimization of the compound was performed through DFT calculations at the B3LYP level combined with 6-311G (d,p) basis sets using Gaussian 09 program.[2]

## 2. Synthesis and Characterization




**Scheme S1:** Synthetic scheme of transporters **1a-1e**.

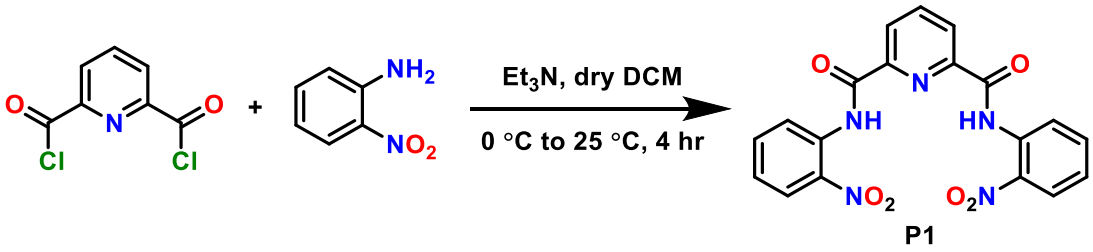
### 2.1 *Synthesis of Pyridine-2,6-dicarbonyl dichloride*




**Scheme S2:** Synthesis of Pyridine-2,6-dicarbonyl dichloride.

In a 25 mL round bottom flask, pyridine-2,6-dicarboxylic acid (500 mg, 3 mmol) was taken, and a catalytic amount (2 drops) of DMF was added. Then 4 mL of thionyl chloride was added, and the mixture was refluxed for 12 hr at 80 °C. After completion of the reaction, the excess $SOCl_2$ was removed under reduced pressure resulting in white solid, which was further used without purification.

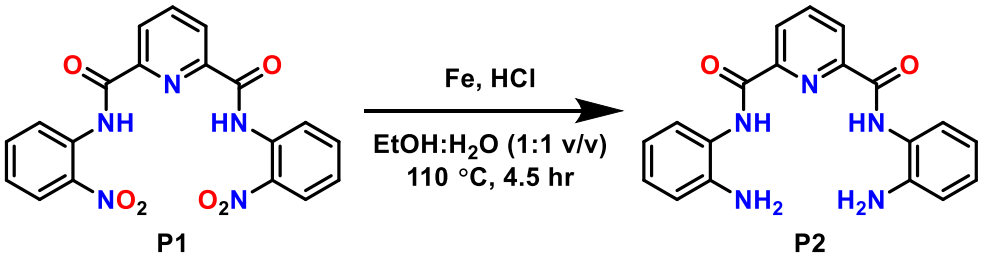
### 2.2 *Synthesis of $N^2$,$N^6$-bis(2-nitrophenyl)pyridine-2,6-dicarboxamide (P1)*




**Scheme S3:** Synthesis of precursor **P1**.

In a 100 mL round bottom flask, 2-nitroaniline (830.62 mg, 6.02 mmol) was taken and diluted with 20 mL of dry DCM and 0.5 mL triethylamine under $N_2$ atmosphere. In a separate round bottom flask, pyridine-2,6-dicarbonyl dichloride (3 mmol) was diluted with 8 mL dry DCM and then dropwise added to the 2-nitroaniline solution at 0 °C. A pale yellow-colored precipitate formed instantly after the addition of the acid chloride. The reaction was continued for 4 hr at 25 °C, and the completion of the reaction was monitored by TLC. The reaction mixture was filtered and washed with cold DCM multiple times, then dried and sent for $^1$H NMR characterization (Yield = 95 %). **$^1$H NMR** (500 MHz, $CDCl_3$): $\delta_{ppm}$ = 12.49 (s, J = 19.3 Hz, 2H), 8.94 (dd, J = 8.4, 1.1 Hz, 2H), 8.56 (d, J = 7.8 Hz, 2H), 8.32 (dd, J = 8.4, 1.4 Hz, 2H), 8.24 (t, J = 7.8 Hz, 1H), 7.80 – 7.76 (m, 2H), 7.34 – 7.30 (m, 2H); **$^{13}$C NMR** (125 MHz, $CDCl_3$) : $\delta_{ppm}$ = 162.07 (s), 148.86 (s), 139.89 (s), 137.65 (s), 135.73 (s), 134.00 (s), 126.17 (s), 124.10 (s), 122.71 (s). **HRMS** (ESI) Calcd. for $C_{19}H_1N_5O_6$ $(M+Na)^+$: 430.08, Found: 430.0757.

### 2.3 *Synthesis of $N^2$,$N^6$-bis(2-aminophenyl)pyridine-2,6-dicarboxamide (P2)*

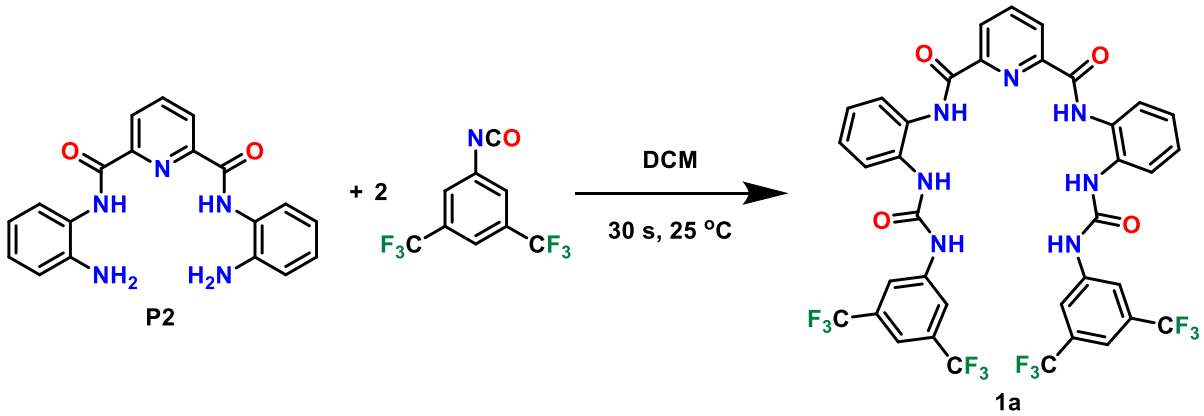



**Scheme S4:** Synthesis of precursor **P2**.

In a 100 mL round bottom flask, precursor **P1** (2.035 g, 5 mmol) was diluted with a 1:1 ratio EtOH: $H_2O$ (60 mL). Then 1.396 g (25 mmol) iron and a catalytic amount of HCl were added and refluxed at 110 °C. The progress of the reaction was monitored through TLC. After 4.5 hr, the reaction mixture was cooled and passed through a celite column. The filtrate was then extracted with EtOAc/$H_2O$, and the organic layer was dried over sodium sulphate. The

solvent was evaporated under reduced pressure to get the pure product (Yield = 78%). **$^{1}$H NMR** (500 MHz, $CDCl_3$): $\delta_{ppm}$ = 9.80 (s, 1H), 8.47 (d, J = 7.8 Hz, 1H), 8.12 (t, J = 7.8 Hz, 1H), 7.61 (dd, J = 7.9, 1.1 Hz, 1H), 7.09 (td, J = 7.7, 1.4 Hz, 1H), 6.95 – 6.82 (m, 2H), 3.94 (s, 2H); **$^{13}$C NMR** (125 MHz, $CDCl_3$): $\delta_{ppm}$ = 161.44, 148.68, 139.71, 139.48, 127.00, 125.47, 124.65, 124.44, 120.28, 118.63; **HRMS** (ESI) Calcd. for $C_{19}H_{17}N_5O_2$ $(M+Na)^+$: 370.1280, Found: 370.1277.

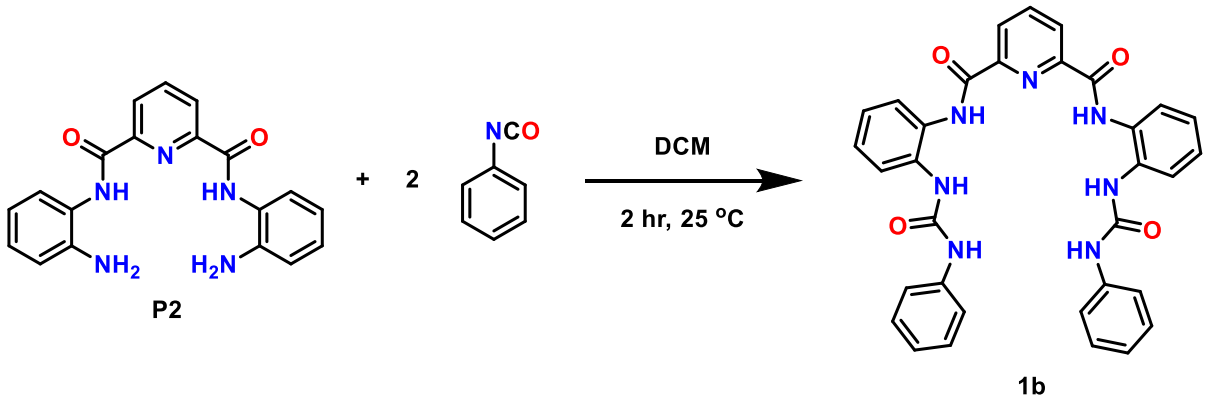
### 2.4 *Synthesis of $N^2$,$N^6$-bis(2-(3-(3,5-bis(trifluoromethyl)phenyl)ureido)phenyl)pyridine-2,6-dicarboxamide (1a)*




**Scheme S5:** Synthesis of Compound **1a**.

In a 25 mL round bottom flask, precursor **P2** (200 mg, 0.57 mmol) and 3,5-bis(trifluoromethyl)phenyl isocyanate (200 µL, 1.16 mmol) were added to 15 mL DCM at 25 °C. A white-colored precipitate formed within 10 sec, and the reaction was stopped after 30 sec by checking the TLC. The precipitate was filtered, washed with DCM, and then dried. It was then sent for $^{1}$H NMR characterization (Yield = 95%). **$^{1}$H NMR** (500 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 11.04 (s, 1H), 9.96 (s, 1H), 8.45 (d, J = 7.2 Hz, 1H), 8.37 (dd, J = 8.6, 6.8 Hz, 1H), 8.10 (s, 1H), 8.06 – 7.99 (m, 1H), 7.63 (s, 1H), 7.32 (dd, J = 7.3, 5.4 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H); **$^{13}$C NMR** (125 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 163.17, 152.87, 148.86, 142.18, 135.10, 131.37, 131.11, 127.98, 127.85, 127.66, 125.90, 124.80, 123.77, 122.94, 122.63, 118.08; **HRMS** (ESI) Calcd. for $C_{37}H_{23}F_{12}N_7O_4$ $(M+Na)^+$: 880.1512, Found: 880.1508.

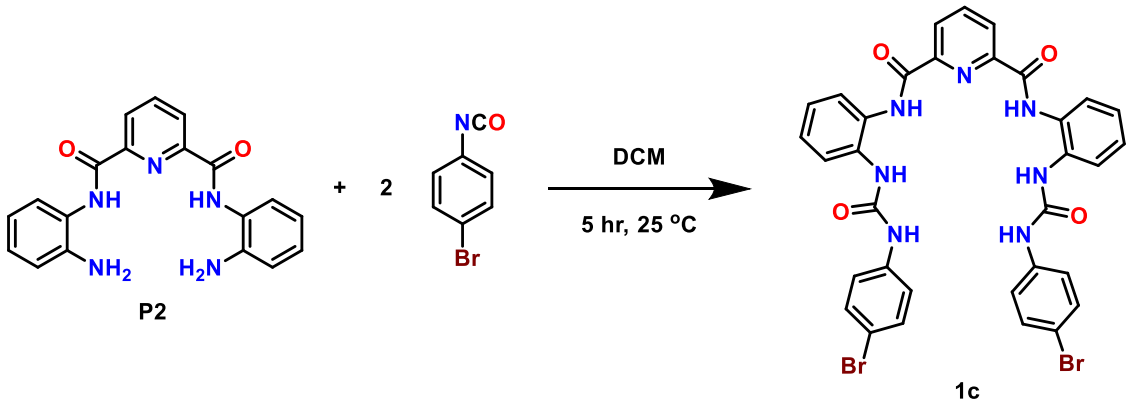
## 2.5 *Synthesis of $N^2$,$N^6$-bis(2-(3-phenylureido)phenyl)pyridine-2,6-dicarboxamide (1b)*




**Scheme S6:** Synthesis of Compound **1b**.

In a 25 mL round bottom flask, precursor **P2** (200 mg, 0.57 mmol) and phenyl isocyanate (127 µL, 1.16 mmol) were added to 15 mL DCM at 25 °C. A white-colored precipitate was formed after 5 minutes. The progress of the reaction was monitored through TLC. After 2 hr, the reaction was stopped, and the precipitate was filtered, washed with DCM, and dried. It was then sent for $^1$H NMR characterization (Yield = 88%). **$^1$H NMR** (500 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 11.00 (s, 1H), 9.21 (s, 1H), 8.44 (d, J = 7.9 Hz, 1H), 8.38 – 8.33 (m, 1H), 8.08 (d, J = 8.2 Hz, 1H), 8.02 (s, 1H), 7.40 (d, J = 8.1 Hz, 2H), 7.34 – 7.28 (m, 2H), 7.24 (t, J = 7.8 Hz, 2H), 7.01 (t, J = 7.6 Hz, 1H), 6.95 (t, J = 7.3 Hz, 1H); **$^{13}$C NMR** (125 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 163.12, 153.05, 149.05, 140.48, 140.08, 136.04, 129.28, 128.09, 127.56, 127.14, 125.74, 122.89, 122.31, 121.98, 118.57; **HRMS** (ESI) Calcd. for $C_{33}H_{27}N_7O_4$ $(M+H)^+$: 586.2197, Found: 586.2178.

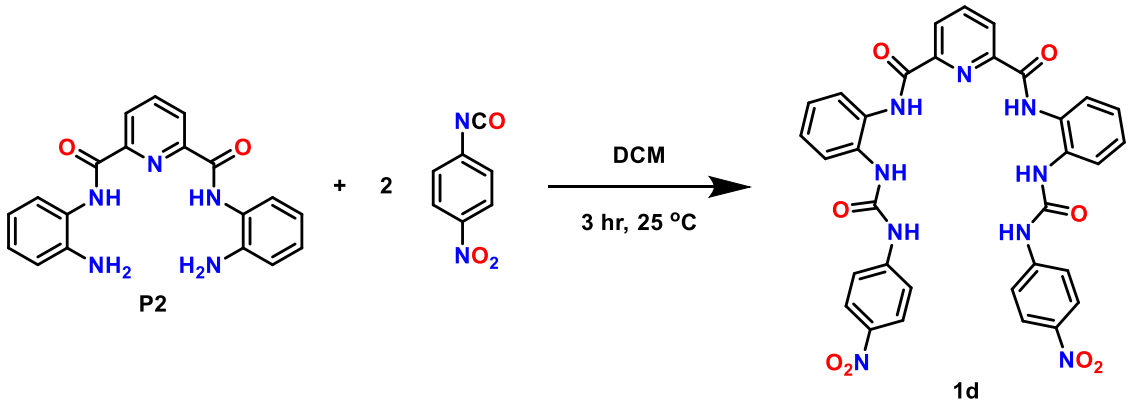
## 2.6 *Synthesis of $N^2$,$N^6$-bis(2-(3-(4-bromophenyl)ureido)phenyl)pyridine-2,6-dicarboxamide (1c)*




**Scheme S7:** Synthesis of Compound **1c**.

In a 50 mL round bottom flask, 4-bromophenyl isocyanate (233.8 mg, 1.16 mmol) was dissolved in 10 mL DCM. In a separate round bottom flask, precursor P2 (200 mg, 0.57 mmol) was dissolved in 20 mL DCM and added to the above isocyanate solution dropwise at 25 °C. A white-colored precipitate was formed after 5 minutes. The progress of the reaction was monitored through TLC. After 5 hr, the reaction was stopped, and the precipitate was filtered, washed with DCM, and dried. It was then sent for $^{1}$H NMR characterization (Yield = 97%). **$^{1}$H NMR** (500 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 10.98 (s, 1H), 9.34 (s, 1H), 8.43 (d, J = 7.5 Hz, 1H), 8.38 – 8.33 (m, 1H), 8.04 (d, J = 6.1 Hz, 2H), 7.38 (q, J = 8.9 Hz, 4H), 7.30 (t, J = 8.9 Hz, 2H), 7.04 (t, J = 7.6 Hz, 1H); **$^{13}$C NMR** (125 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 163.08, 152.93, 148.99, 140.51, 139.49, 135.71, 132.00, 128.01, 127.56, 127.34, 125.78, 123.14, 122.19, 120.46, 113.70; **HRMS** (ESI) Calcd. for $C_{33}H_{25}Br_2N_7O_4$ $(M+Na)^+$: 764.0227, Found: 764.0200.

### 2.7 *Synthesis of $N^2$,$N^6$-bis(2-(3-(4-nitrophenyl)ureido)phenyl)pyridine-2,6-dicarboxamide (1d)*

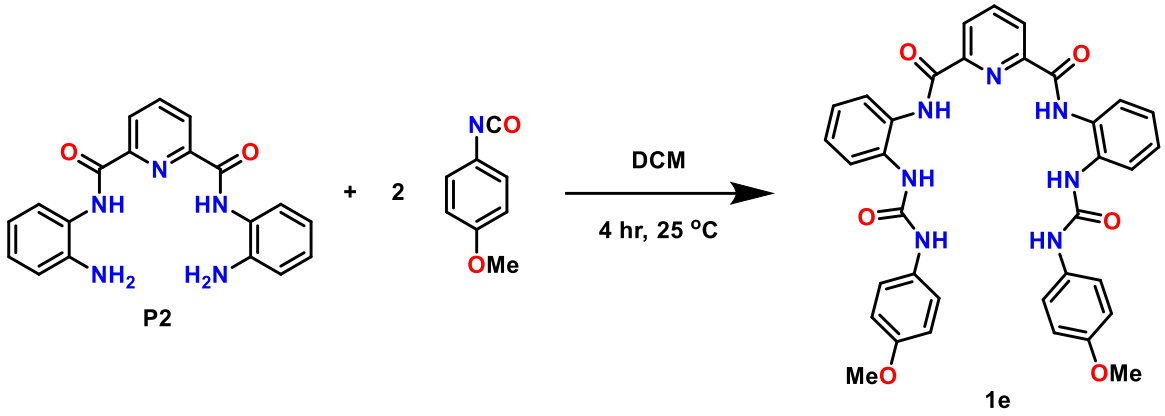



**Scheme S8:** Synthesis of Compound **1d**.

In a 50 mL round bottom flask, 4-nitrophenyl isocyanate (190 mg, 1.16 mmol) was dissolved in 10 mL DCM. In a separate round bottom flask, precursor P2 (200 mg, 0.57 mmol) was dissolved in 20 mL DCM and added to the above isocyanate solution dropwise at 25 °C. A white-colored precipitate was formed after 5 minutes. The progress of the reaction was monitored through TLC. After 5 hr, the reaction was stopped, and the precipitate was filtered, washed with DCM, and dried. It was then sent for $^{1}$H NMR characterization (Yield = 98%). **$^{1}$H NMR** (500 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 11.00 (s, 2H), 9.90 (s, 2H), 8.45 (d, J = 7.7 Hz, 2H), 8.39 – 8.35 (m, 1H), 8.21 (s, 2H), 8.13 (d, J = 9.2 Hz, 4H), 8.00 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 9.2 Hz, 4H), 7.33 (dd, J = 18.8, 7.8 Hz, 4H), 7.07 (t, J = 7.2 Hz, 2H); **$^{13}$C NMR** (125 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 163.01, 152.58, 148.91, 146.67, 141.47, 140.61, 135.03, 127.90, 127.79, 127.57,

125.86, 125.61, 123.75, 122.64, 117.82; **HRMS** (ESI) Calcd. for $C_{33}H_{25}N_9O_8$ $(M+Na)^+$: 698.1718, Found: 698.1704.

### 2.8 *Synthesis of $N^2$,$N^6$-bis(2-(3-(4-methoxyphenyl)ureido)phenyl)pyridine-2,6-dicarboxamide (1e)*




**Scheme S9:** Synthesis of Compound **1e**.

In a 25 mL round bottom flask, precursor **P2** (200 mg, 0.57 mmol) and 4-methoxyphenyl isocyanate (150 µL, 1.16 mmol) were added to 15 mL DCM at 25 °C. A white-colored precipitate was formed after 5 minutes. The progress of the reaction was monitored through TLC. After 2 hr, the reaction was stopped, and the precipitate was filtered, washed with DCM, and dried. It was then sent for $^1$H NMR characterization (Yield = 90%). **$^1$H NMR** (500 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 10.97 (s, 2H), 9.00 (s, 2H), 8.43 (d, J = 7.7 Hz, 2H), 8.38 – 8.33 (m, 1H), 8.06 (d, J = 8.2 Hz, 2H), 7.95 (s, 2H), 7.33 (d, J = 7.7 Hz, 2H), 7.29 (t, J = 9.2 Hz, 6H), 7.04 (t, J = 7.5 Hz, 2H), 6.81 (d, J = 8.8 Hz, 4H); **$^{13}$C NMR** (125 MHz, DMSO-$d_6$): $\delta_{ppm}$ = 163.10, 154.95, 153.26, 149.07, 140.45, 136.05, 132.99, 127.97, 127.51, 127.12, 125.72, 122.77, 121.91, 120.46, 114.46, 55.59; **HRMS** (ESI) Calcd. for $C_{35}H_{31}N_7O_6$ $(M+H)^+$: 646.2409, Found: 646.2396.

## 3. Anion Binding Studies

### *3.1 Anion Screening by $^1$H NMR Spectroscopy*

For anion binding studies, $^1$H NMR titrations were performed for Compound **1a** in DMSO-$d_6$/0.5% $H_2O$ solvent system in Bruker's Ultra Shield (500 MHz) spectrometer. Tetrabutylammonium (TBA) salts of different anions ($Cl^-$, $Br^-$, $I^-$, $NO_3^-$, $ClO_4^-$, and $AcO^-$) were used for the screening. NMR samples were prepared by mixing Compound **1a** (10 mM) with different TBA salts (5 eq.) in DMSO-$d_6$ solvent. Compound **1a** shows a selective response to TBACl and TBAOAc, indicated by deshielding in the urea N-H protons.

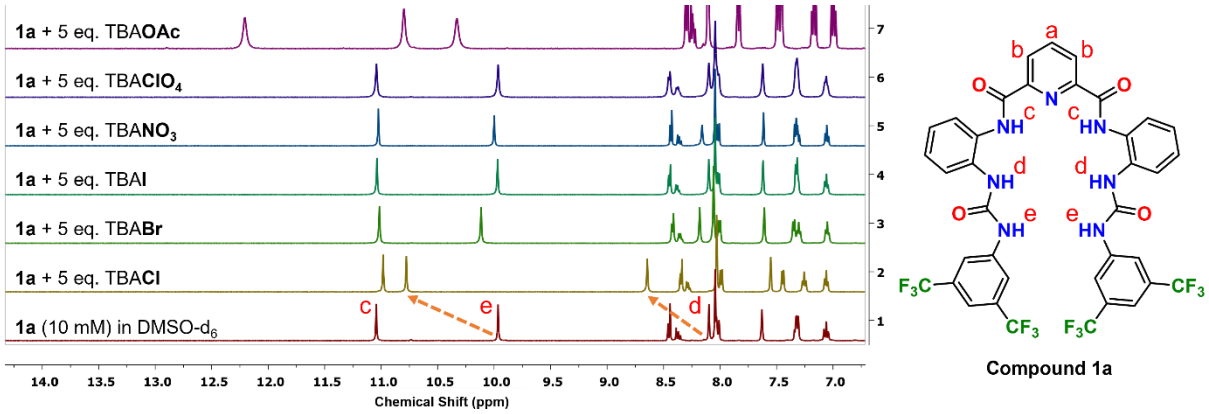



**Fig. S1** Stacked $^1$H NMR plot of change in chemical shift of N-H protons of **1a** with different TBA salts in DMSO-$d_6$/0.5% $H_2O$.

### *3.2 Determination of Binding Stoichiometry by Job's Plot from $^1$H NMR Titration*

The binding stoichiometry between the compound and the anion was calculated using the continuous variation method of Job's plot experiment. For the experiment, the stock solutions of Compound **1a** (10 mM) and TBACl (10 mM) were prepared in DMSO-$d_6$ solvent. During the experiment, 10 separate NMR tubes were prepared, taking different molar ratios of the host and guest, as mentioned in Table S1, and sent for $^1$H NMR. The chemical shift in the urea N-$H_e$ proton at different mole fractions was taken for calculation (Table S1). The Job's plot is shown in Fig. S2, which clearly shows 1:1 binding stoichiometry.

**Table S1.** Result and Calculation table for Job's plot experiment.

| Sl. No. | Host Conc. ([H], mM) | Guest Conc. ([G], mM) | [H] + [G] | $\frac{[H]}{[H]+[G]}$ | δ of N-$H_e$ proton | Δδ | $\left(\frac{[H]}{[H]+[G]}\right) * \Delta\delta$ |
|---|---|---|---|---|---|---|---|
| **1** | 1.0 | 9.0 | 10.0 | 0.1 | 10.4387 | 0.4777 | 0.04777 |
| **2** | 2.0 | 8.0 | 10.0 | 0.2 | 10.3974 | 0.4364 | 0.08728 |
| **3** | 3.0 | 7.0 | 10.0 | 0.3 | 10.3504 | 0.3894 | 0.11682 |
| **4** | 4.0 | 6.0 | 10.0 | 0.4 | 10.2968 | 0.3358 | 0.13432 |
| **5** | 5.0 | 5.0 | 10.0 | 0.5 | 10.2435 | 0.2825 | 0.14125 |
| **6** | 6.0 | 4.0 | 10.0 | 0.6 | 10.1898 | 0.2288 | 0.13728 |
| **7** | 7.0 | 3.0 | 10.0 | 0.7 | 10.1306 | 0.1696 | 0.11872 |
| **8** | 8.0 | 2.0 | 10.0 | 0.8 | 10.074 | 0.1130 | 0.09040 |
| **9** | 9.0 | 1.0 | 10.0 | 0.9 | 10.0176 | 0.0566 | 0.05094 |
| **10** | 10.0 | 0.0 | 10.0 | 1.0 | 9.961 | 0 | 0 |

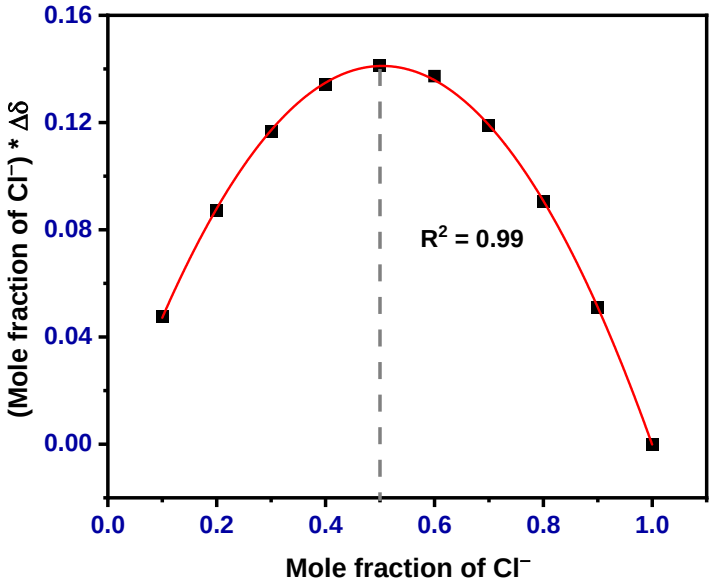



**Fig. S2** Job's plot for Compound **1a** and TBACl obtained from $^1$H NMR titration experiment in DMSO-$d_6$/0.5% $H_2O$ (500 MHz, 25 °C).

### *3.3 Binding Stoichiometry by ESI-MS*

In order to confirm the binding stoichiometry of the chloride-bound complex in solution form, an ESI-MS experiment was performed in negative scan mode. The sample was prepared by adding 2 equivalents of TBACl into a solution of Compound **1a** (1 mM) in methanol. The mass spectrum provided an intense signal peaked at m/z = 892.1284, corresponding to the [**1a**+ $Cl^-$] complex, thus confirming the formation of a 1:1 complex in solution phase (Fig. S3).

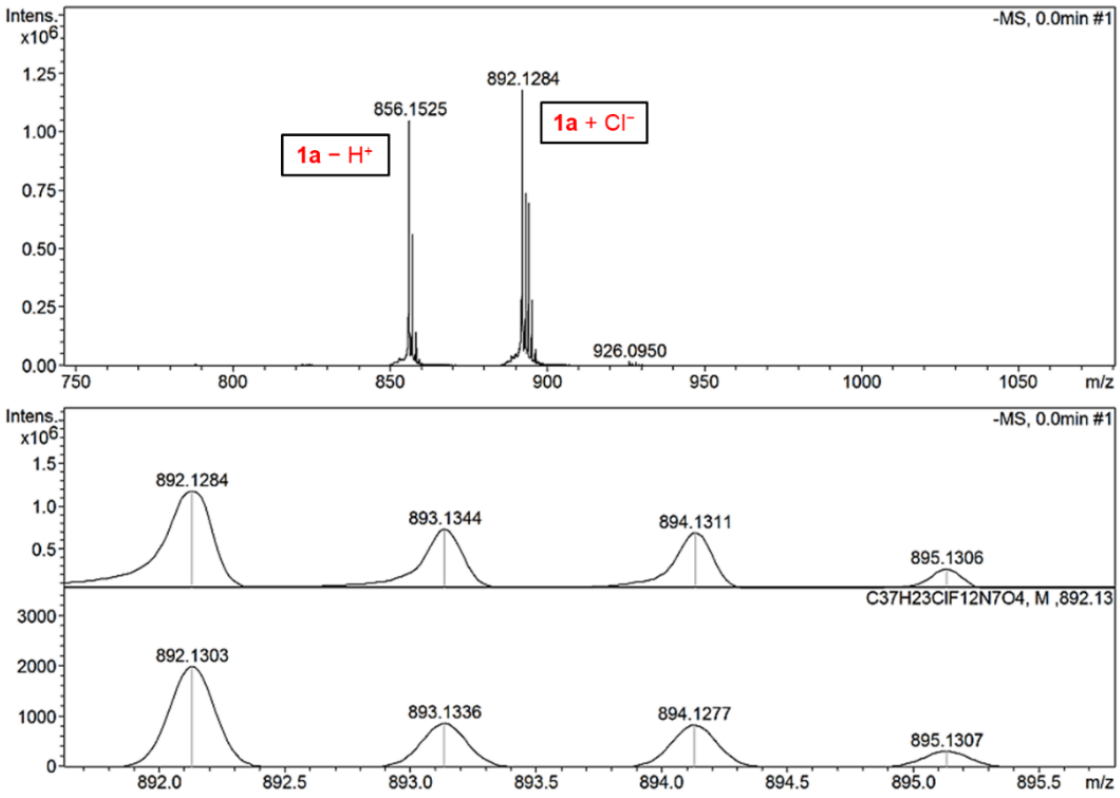
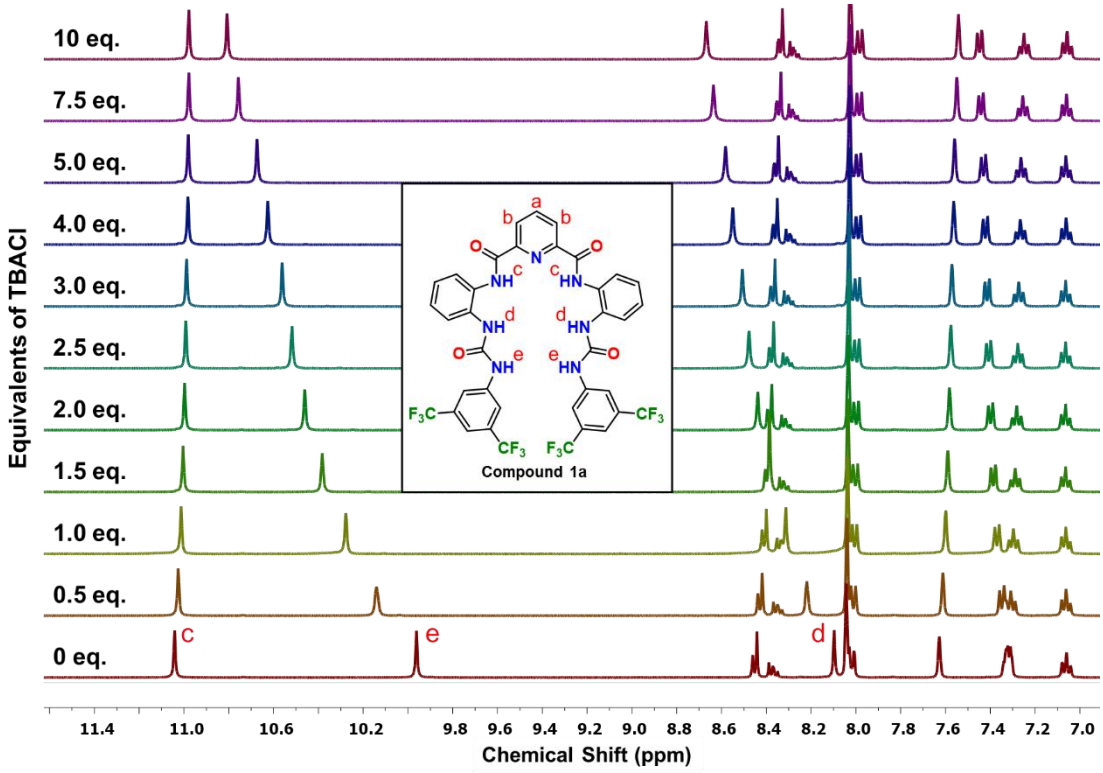
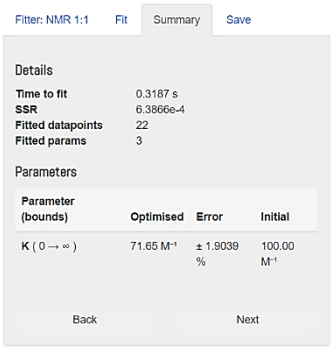



**Fig. S3** ESI-MS spectrum of Compound **1a** (1 mM) with 2 equivalents of TBACl in negative scan mode.

### *3.4 $^{1}$H NMR Titration Studies*

The stock solutions of Compounds **1a-1e** (10 mM) and tetrabutylammonium salts of different anions ($Cl^-$, $Br^-$, $I^-$, $NO_3^-$, $ClO_4^-$, and $AcO^-$) (500 mM) were prepared in DMSO-$d_6$ solvent. $^{1}$H NMR spectra were recorded in Bruker's Ultra Shield (400 MHz) spectrometer. The titrations were performed by stepwise addition of TBA salts of different anions (0–10 eq.) to the solution of Compounds **1a-1e** in DMSO-$d_6$. All the NMR spectra were processed and stacked using the MestReNova software. Furthermore, upon the addition of different TBA salts, the change in the chemical shift of urea N-$H_d$ and N-$H_e$ peaks against the concentration of guest added were fitted to a 1:1 and 1:2 (for $AcO^-$) binding model of BindFit v5.0 program, and the binding constants ($K_a$) were calculated.



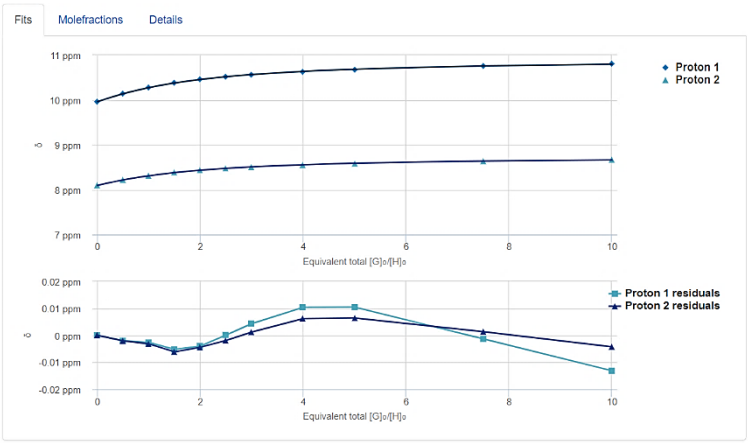
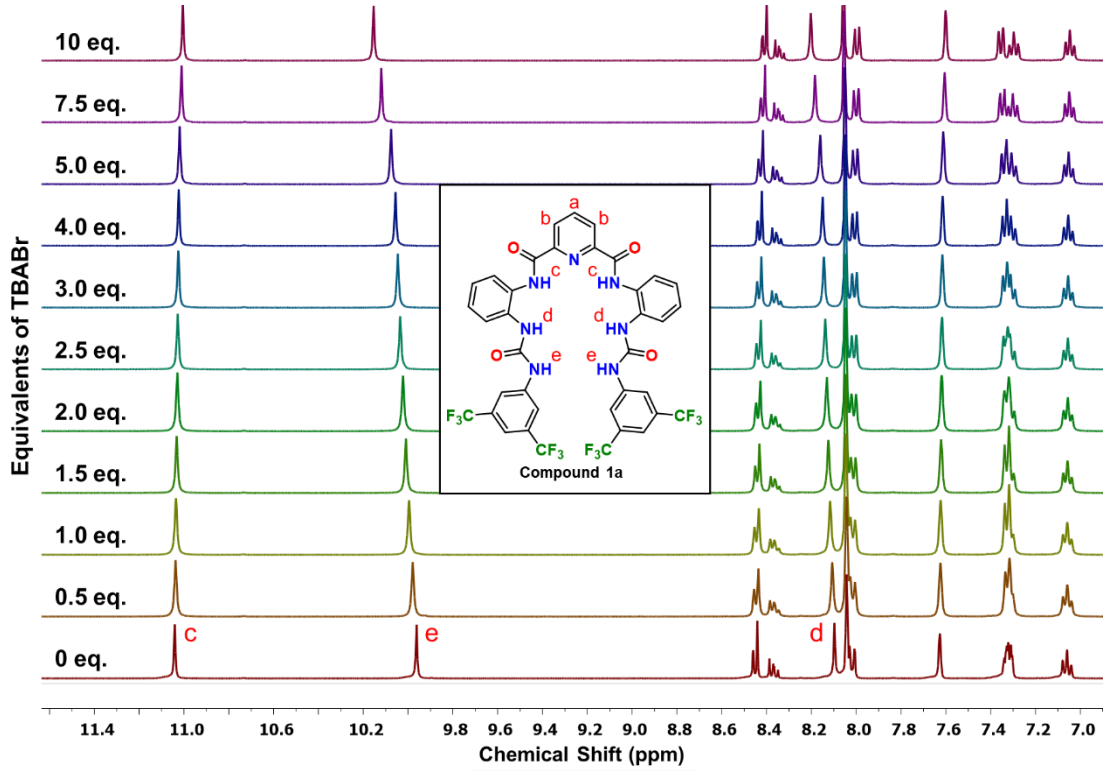




**Fig. S4** (Top) Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1a** with increasing equivalents of TBACl (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C). (Bottom) Screenshot of fitted data using BindFit from supramolecular.org. The data was fitted to a 1:1 binding model using the BindFit v0.5 program and the binding constant ($K_a$) was found to be 71.65 $M^{-1}$ ± 1.90%. The BindFit URL of the experiment is http://app.supramolecular.org/bindfit/view/8fedc700-4525-42dd-b054-f0025897bf3e.



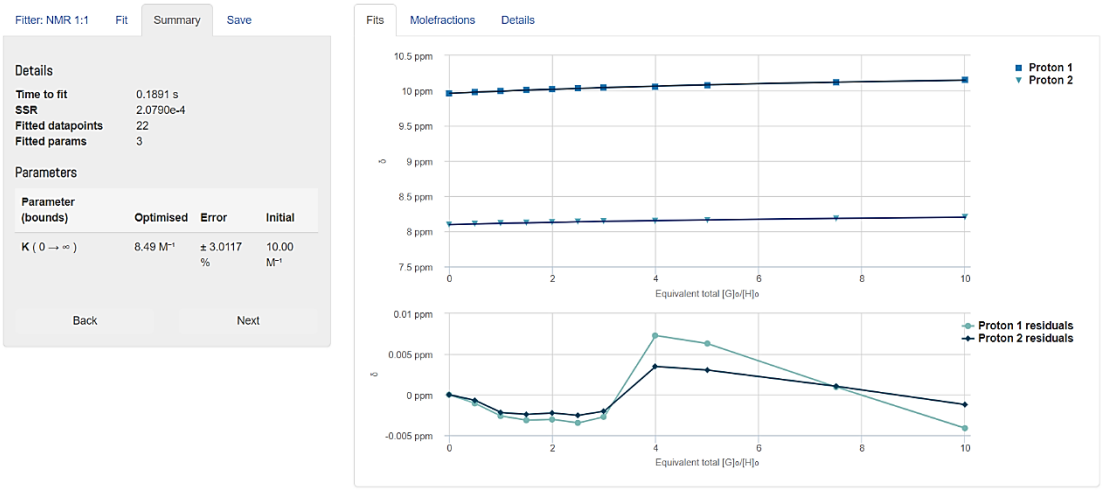
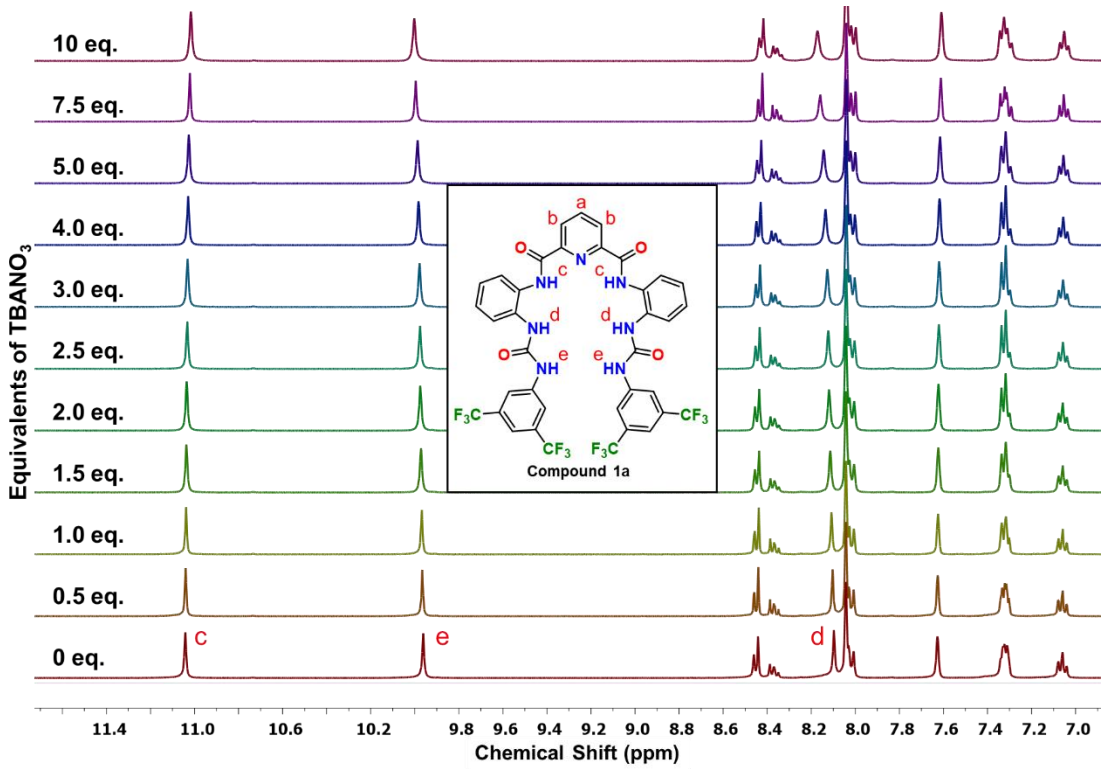




**Fig. S5** (Top) Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1a** with increasing equivalents of TBABr (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C). (Bottom) Screenshot of fitted data using BindFit from supramolecular.org. The data was fitted to a 1:1 binding model using the BindFit v0.5 program and the binding constant ($K_a$) was found to be 8.49 $M^{-1}$ ± 3.01%. The BindFit URL of the experiment is http://app.supramolecular.org/bindfit/view/10ab3e4b-656c-438c-a345-2ca85879ed28.



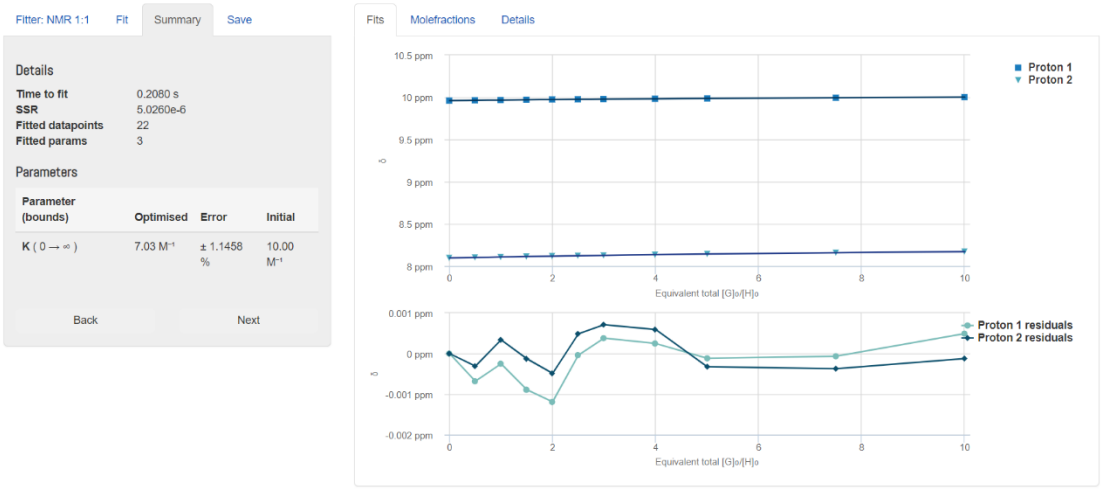




**Fig. S6** (Top) Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1a** with increasing equivalents of $TBANO_3$ (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C). (Bottom) Screenshot of fitted data using BindFit from supramolecular.org. The data was fitted to a 1:1 binding model using the BindFit v0.5 program and the binding constant ($K_a$) was found to be 7.03 $M^{-1}$ ± 1.14%. The BindFit URL of the experiment is http://app.supramolecular.org/bindfit/view/72f2a949-c1c9-4c66-b22e-1ae59583bd34.

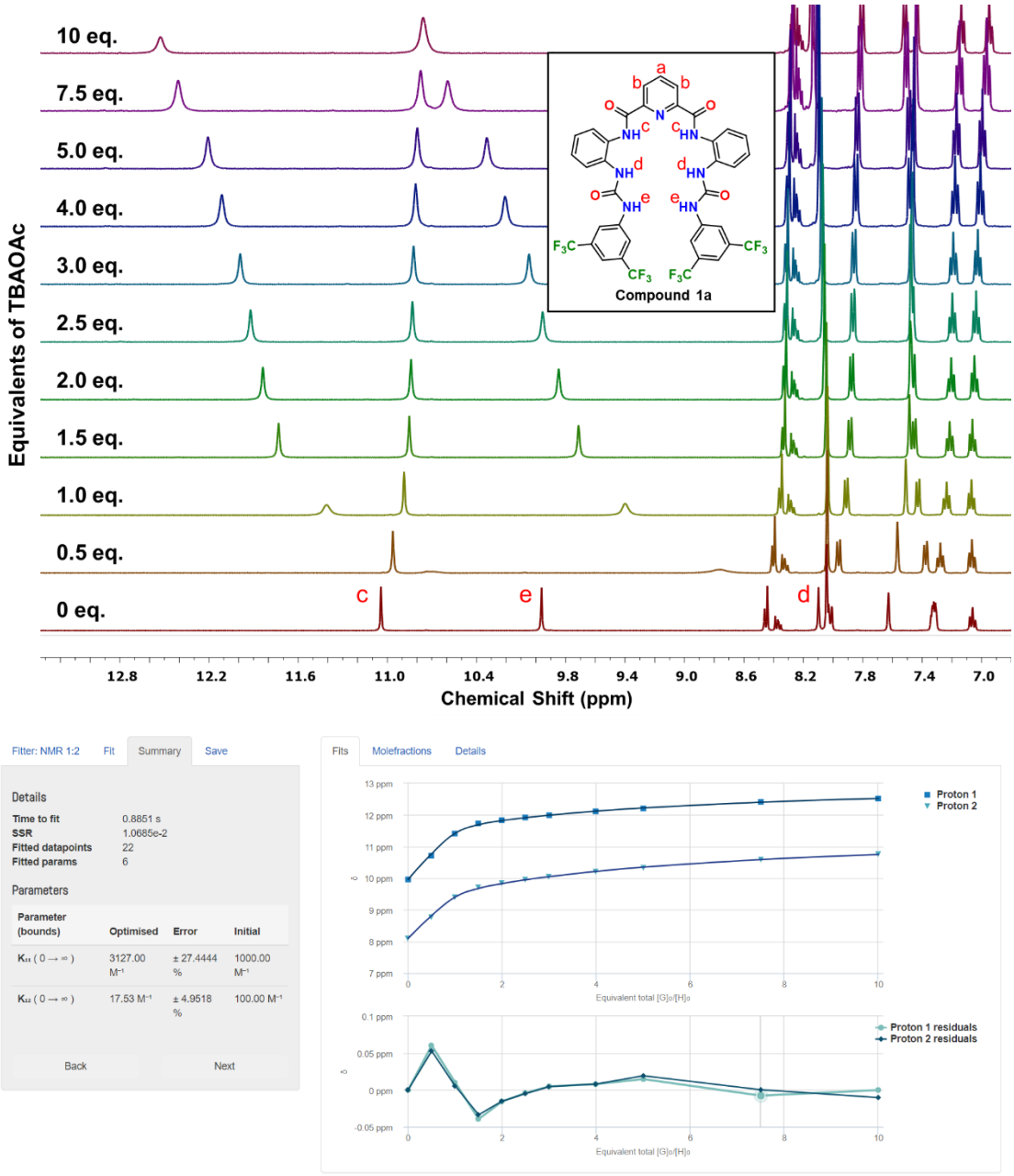



**Fig. S7** (Top) Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1a** with increasing equivalents of TBAOAc (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C). (Bottom) Screenshot of fitted data using BindFit from supramolecular.org. The data was fitted to a 1:2 binding model using the BindFit v0.5 program and the binding constants were found to be $K_{11}$ = 3127.00 $M^{-1}$ ± 27.44%, and $K_{12}$ = 17.53 $M^{-1}$ ± 4.95%. The BindFit URL of the experiment is http://app.supramolecular.org/bindfit/view/dc87c5b4-1b0f-448e-8c16-b30f42393f29.

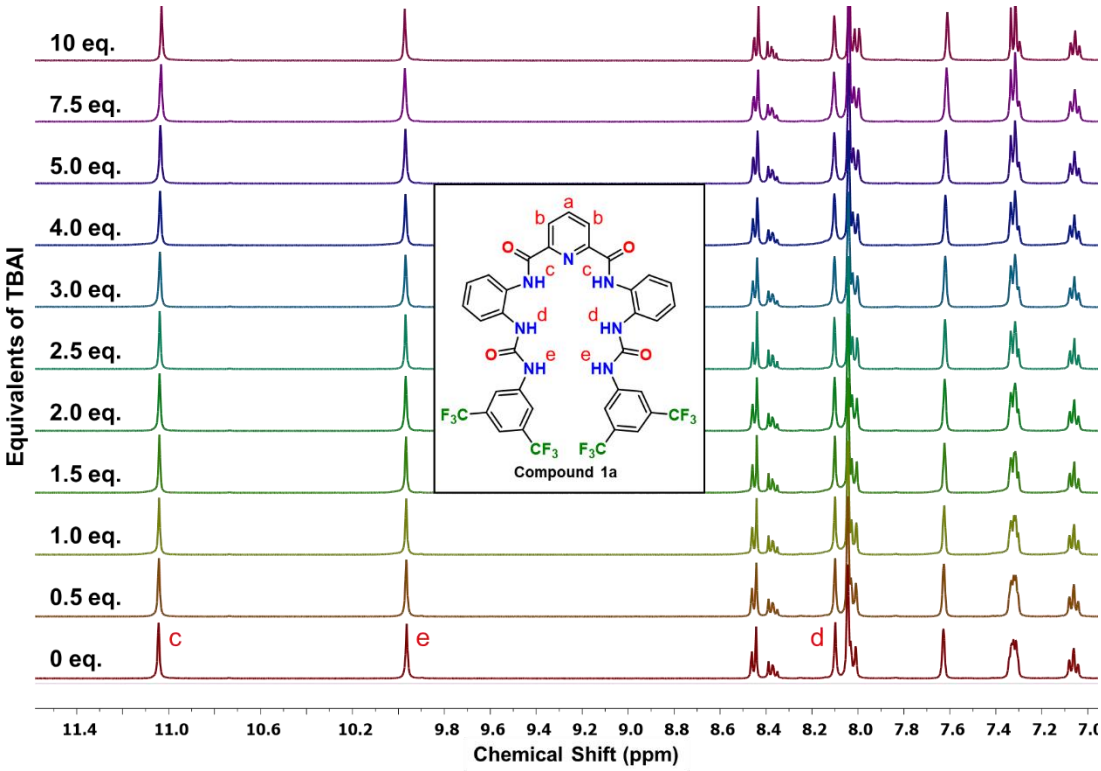



**Fig. S8** Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1a** with increasing equivalents of TBAI (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C).

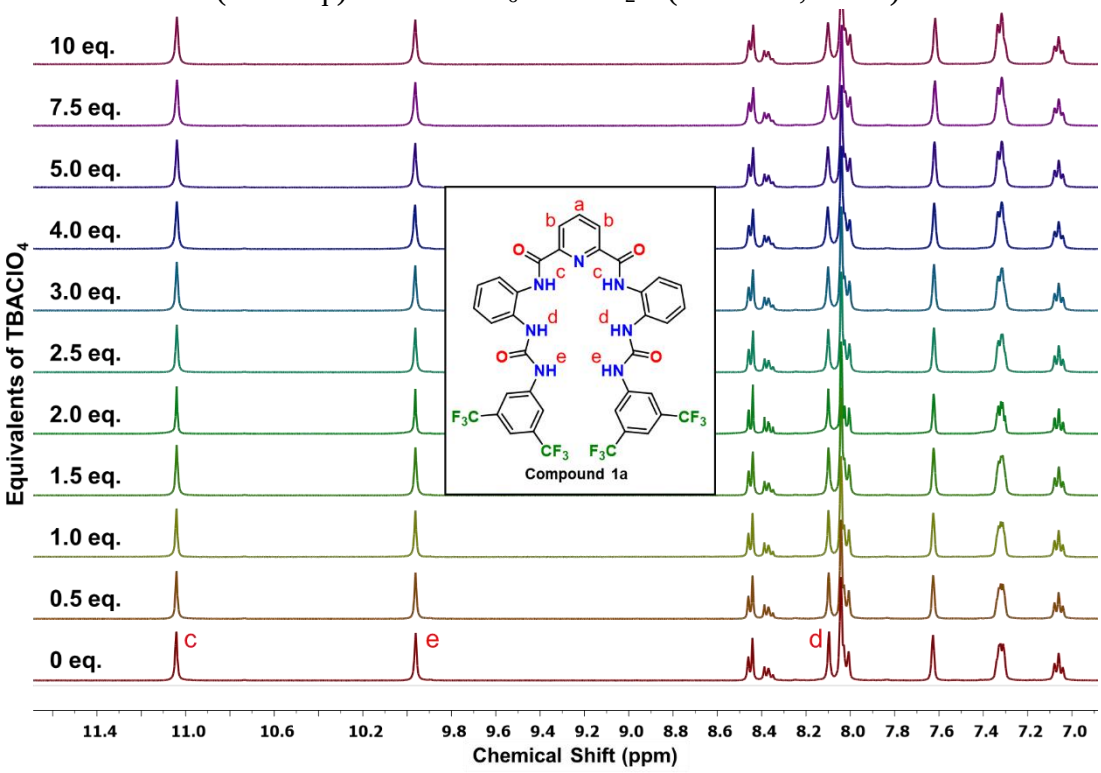
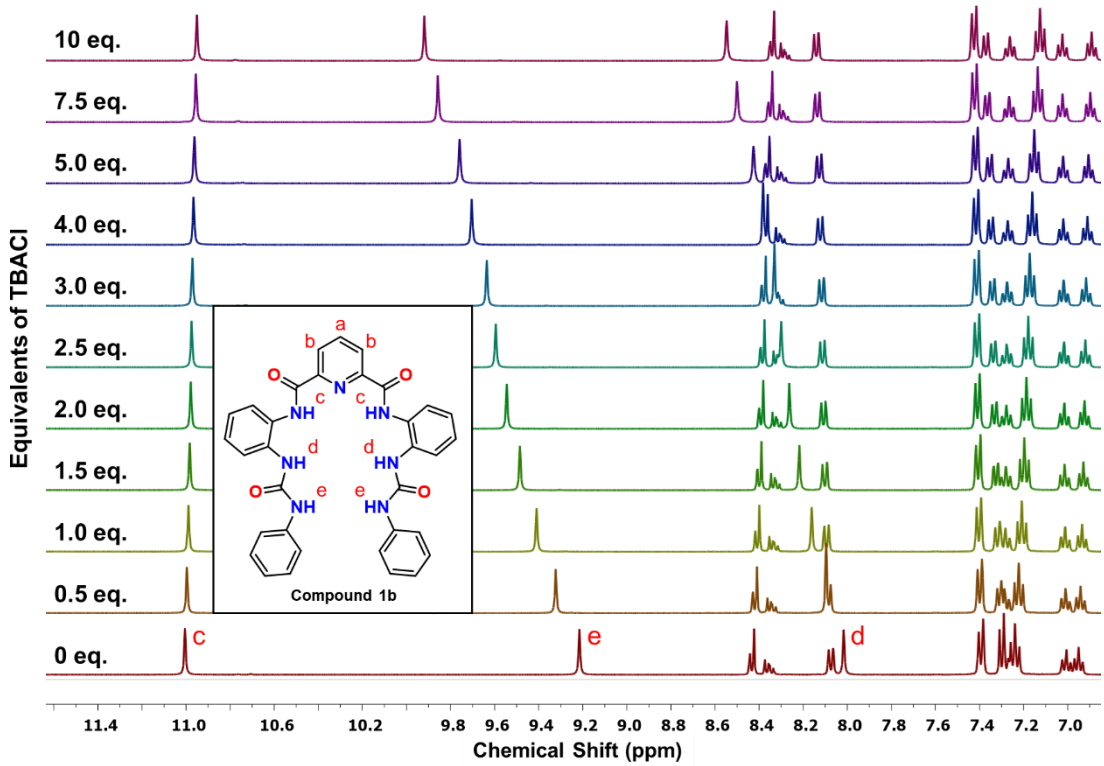



**Fig. S9** Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1a** with increasing equivalents of $TBAClO_4$ (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C).



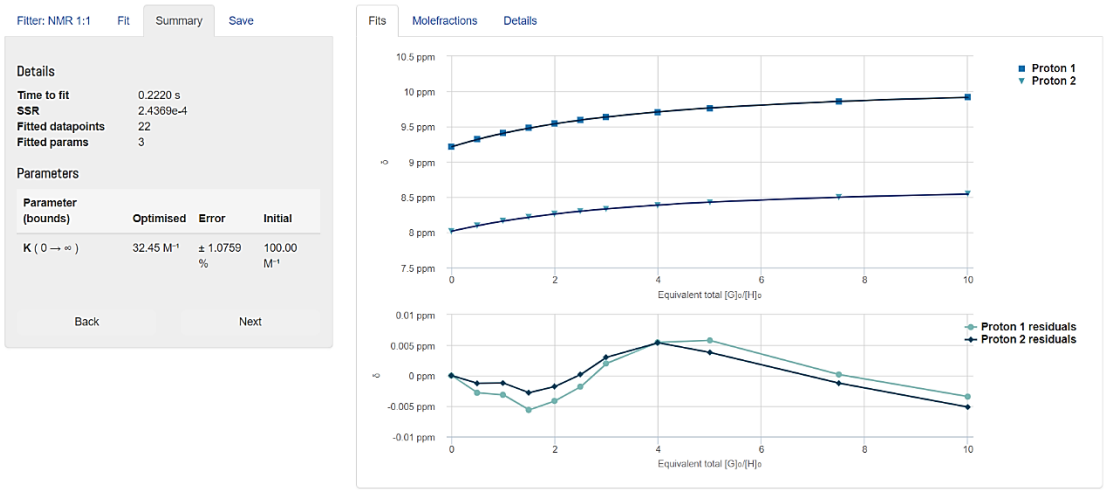
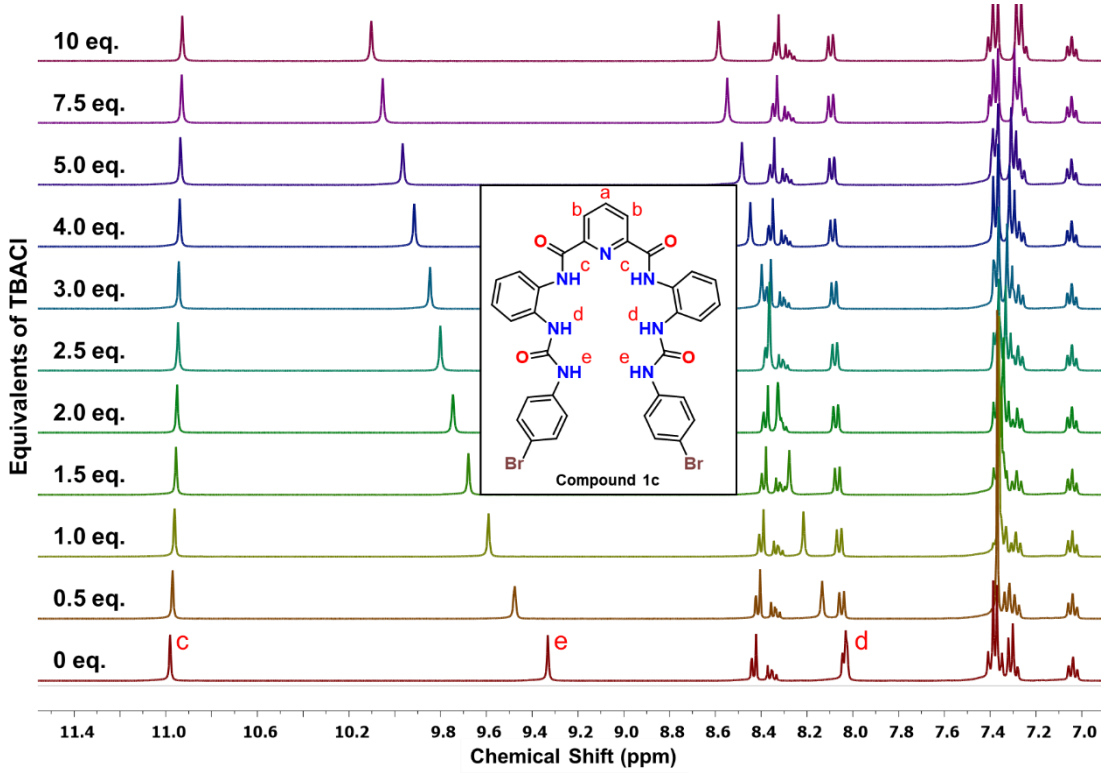




**Fig. S10** (Top) Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1b** with increasing equivalents of TBACl (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C). (Bottom) Screenshot of fitted data using BindFit from supramolecular.org. The data was fitted to a 1:1 binding model using the BindFit v0.5 program and the binding constant ($K_a$) was found to be 32.45 $M^{-1}$ ± 1.07%. The BindFit URL of the experiment is http://app.supramolecular.org/bindfit/view/7ac41ab2-d8e7-4ec3-9310-c8d9f271665f.



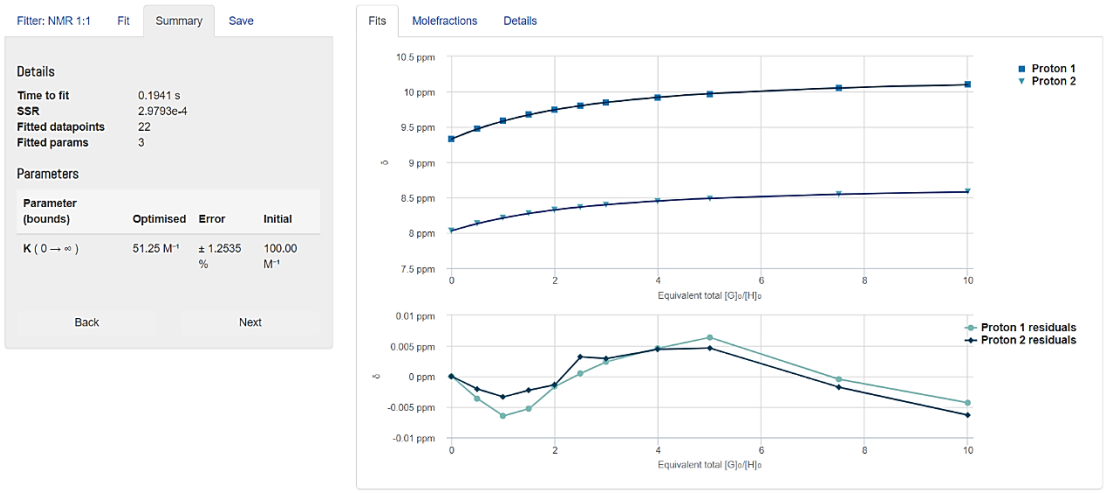
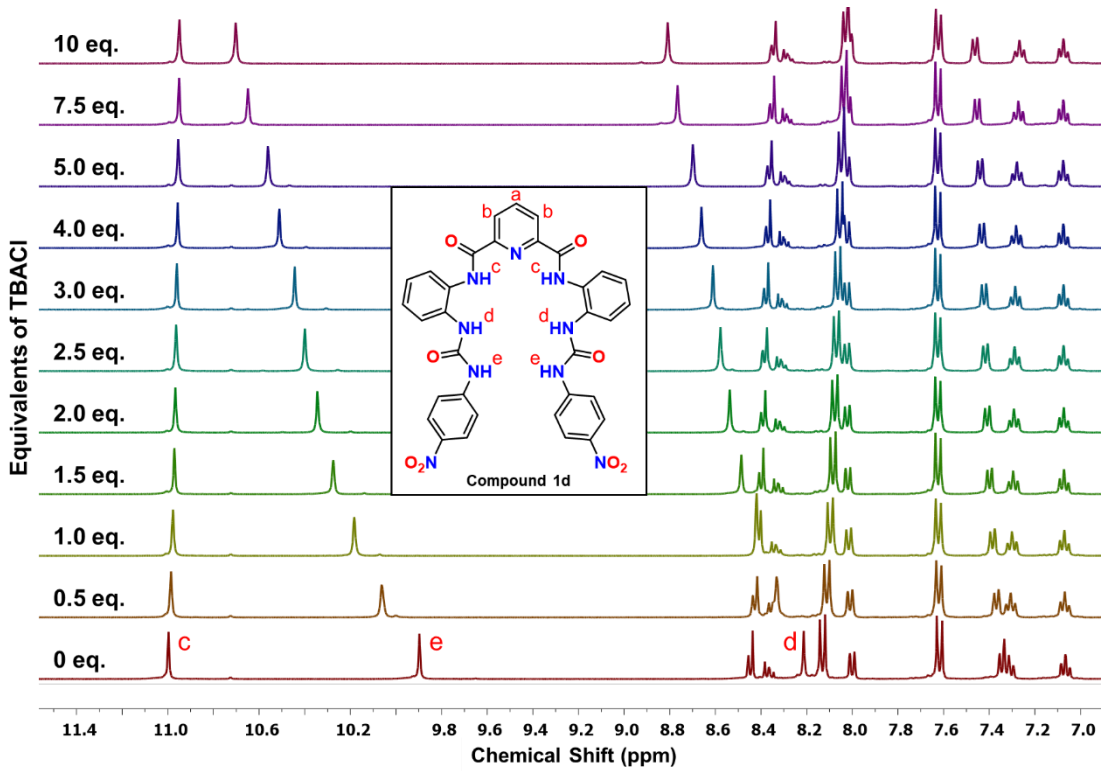
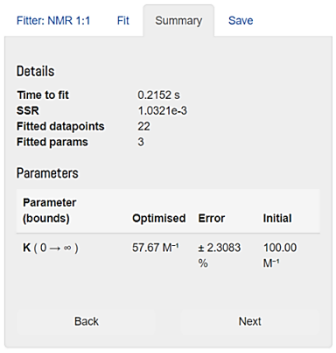




**Fig. S11** (Top) Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1c** with increasing equivalents of TBACl (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C). (Bottom) Screenshot of fitted data using BindFit from supramolecular.org. The data was fitted to a 1:1 binding model using the BindFit v0.5 program and the binding constant ($K_a$) was found to be 51.25 $M^{-1}$ ± 1.25%. The BindFit URL of the experiment is http://app.supramolecular.org/bindfit/view/5e2ab54f-5d7a-4c1d-a7de-0240b7a9edfc.



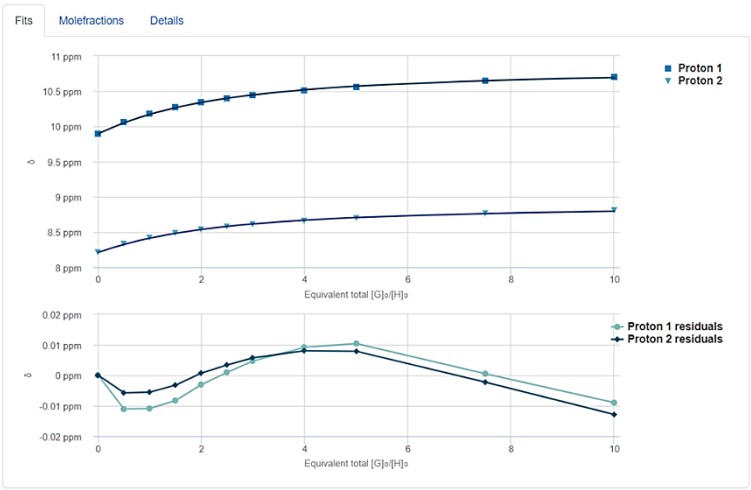




**Fig. S12** (Top) Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1d** with increasing equivalents of TBACl (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C). (Bottom) Screenshot of fitted data using BindFit from supramolecular.org. The data was fitted to a 1:1 binding model using the BindFit v0.5 program and the binding constant ($K_a$) was found to be 57.67 $M^{-1}$ ± 2.30%. The BindFit URL of the experiment is http://app.supramolecular.org/bindfit/view/076d7181-93a3-4f01-8394-b2a67f0e67c0.

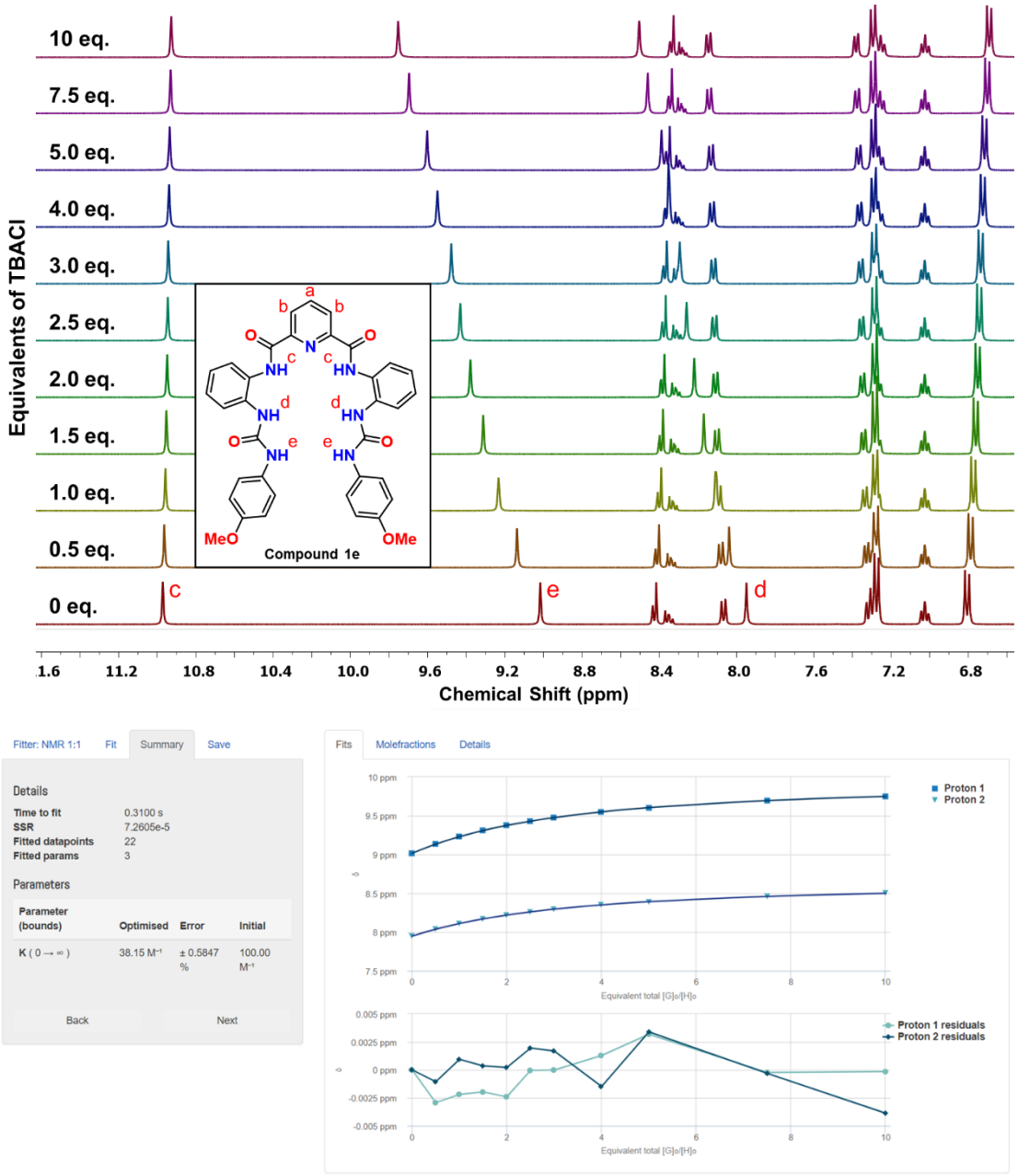
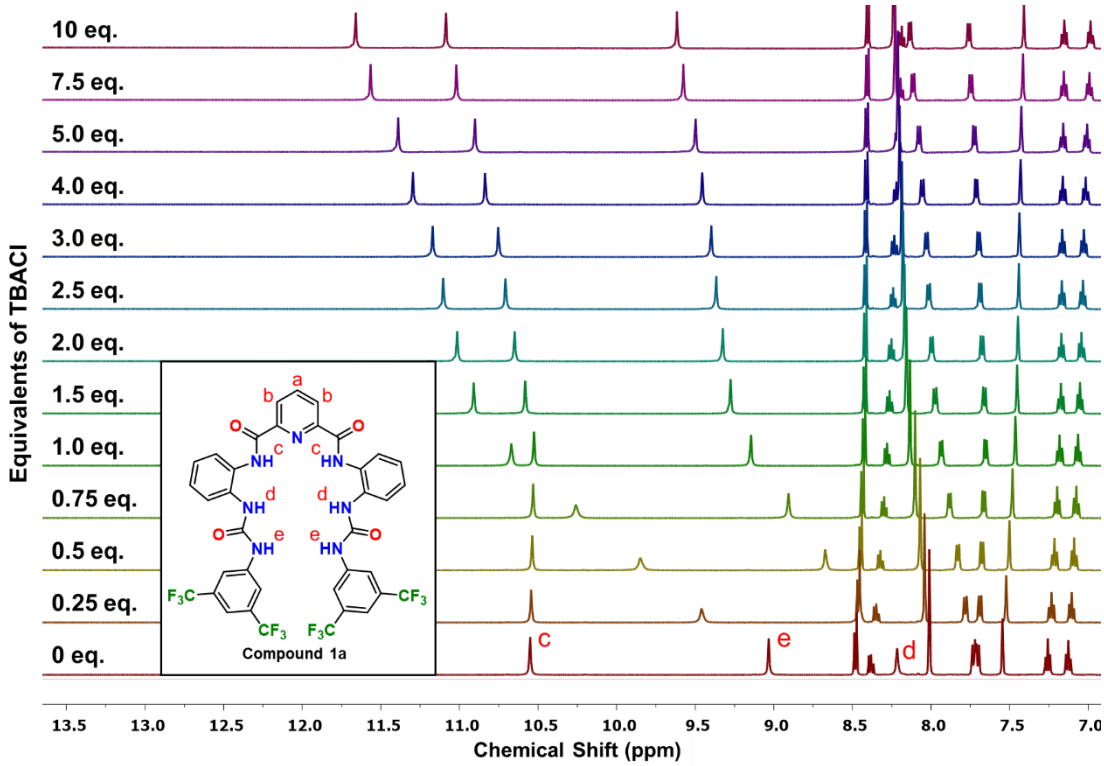
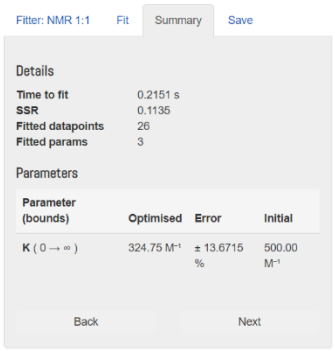



**Fig. S13** (Top) Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1e** with increasing equivalents of TBACl (0–10 eq.) in DMSO-$d_6$/0.5% $H_2O$ (400 MHz, 25 °C). (Bottom) Screenshot of fitted data using BindFit from supramolecular.org. The data was fitted to a 1:1 binding model using the BindFit v0.5 program and the binding constant ($K_a$) was found to be 38.15 $M^{-1}$ ± 0.58%. The BindFit URL of the experiment is http://app.supramolecular.org/bindfit/view/70412437-6814-48a3-9053-d0720287e571.



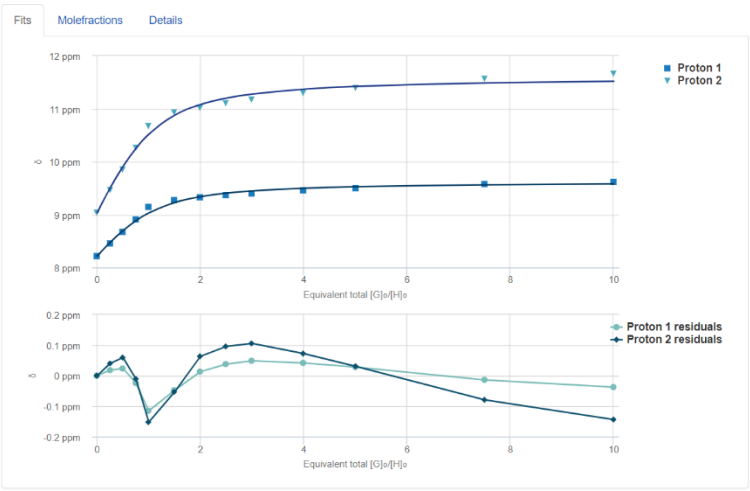




**Fig. S14** (Top) Stacked $^{1}$H NMR plot of change in chemical shift of N-H protons of **1a** with increasing equivalents of TBACl (0–10 eq.) in Acetone-$d_6$ (400 MHz, 25 °C). (Bottom) Screenshot of fitted data using BindFit from supramolecular.org. The data was fitted to a 1:1 binding model using the BindFit v0.5 program and the binding constant ($K_a$) was found to be 324.75 $M^{-1}$ ± 13.67%. The BindFit URL of the experiment is http://app.supramolecular.org/bindfit/view/f6877dc4-2f67-4703-a926-21f0e0d1b145.

**Table S2.** Binding constant ($K_a$) calculation table for Compounds (**1a-1e**).

| Compound | $Cl^-$ | $Br^-$ | $I^-$ | $ClO_4^-$ | $NO_3^-$ | $AcO^-$ |
|---|---|---|---|---|---|---|
| **1a** | 71.65 ± 1.90% | 8.49 ± 3.01% | N.D.[a] | N.D.[a] | 7.03 ± 1.14% | $K_{11}$ = 3127.0 $M^{-1}$ ± 27.44%<br>$K_{12}$ = 17.53 ± 4.95% |
| **1b** | 32.45 ± 1.07% | -[b] | -[b] | -[b] | -[b] | -[b] |
| **1c** | 51.25 ± 1.25% | -[b] | -[b] | -[b] | -[b] | -[b] |
| **1d** | 57.67 ± 2.31% | -[b] | -[b] | -[b] | -[b] | -[b] |
| **1e** | 38.15 ± 0.58% | -[b] | -[b] | -[b] | -[b] | -[b] |

[a] binding constant could not be calculated due to the absence of the change in the chemical shift of urea N-H protons. [b] binding constant was not determined.

### *3.5 Anion Binding and Unbinding Study*

The anion binding and subsequent unbinding of the $Cl^-$ ion to **1a** was investigated through $^1H$ NMR experiments. For the binding experiment, 5 eq. of TBACl was added to **1a** (10 mM) in DMSO-$d_6$/0.5% $H_2O$ and sent for NMR experiment. Then to the TBACl solution of **1a**, 6 eq. of $Ag(SbF_6)$ was added. A white precipitate was formed, which was removed by centrifugation and then sent for NMR experiment.

## 4. Single Crystal X-Ray Diffraction (SC-XRD) Analysis

Single crystals of Compound **1a** were obtained through the vapor diffusion method. Vapors of diethyl ether were diffused into the acetone solution of Compound **1a** over a period of 2 weeks to get suitable crystals for X-ray diffraction. Crystals data were solved and then refined using OLEX2 software using the program SHELXL.

Two molecules of acetone in the asymmetric unit of **1a** could not be modeled satisfactorily and hence removed from the electron density map using the solvent mask command of OLEX2.[3]

### *4.1 Crystallographic Data*

**Table S3.** Crystal data and structure refinement for Compound **1a**⊃$H_2O$.

| | |
|---|---|
| **Formula** | $C_{37}$ $H_{25}$ $F_{12}$ $N_7$ $O_5$ |
| **Formula Weight** | 875.64 |
| **Temperature (K)** | 140 |
| **Crystal System** | Triclinic |
| **Space Group** | P -1 |
| **Z** | 2 |

| | |
|---|---|
| ***a* ( Å )** | 10.587(2) |
| ***b* ( Å )** | 15.112(3) |
| ***c* ( Å )** | 16.143(3) |
| ***α* ( °)** | 107.182(12) |
| ***β* ( °)** | 102.520(13) |
| ***γ* ( °)** | 106.296(11) |
| **Volume (Å$^3$)** | 2238.3(8) |
| **Density (g cm$^{-3}$)** | 1.299 |
| **F (000), μ (mm$^{-1}$)** | 888.0, 0.121 |
| **θ (min, max) ( °)** | 2.423, 27.885 |
| **$h_{min, max}$, $k_{min, max}$, $l_{min, max}$** | (-13, 13), (-19, 19), (-21, 21) |
| **Radiation** | Mo Kα (λ = 0.71073 Å) |
| **Reflections collected** | 10655 |
| **Final R indexes (all data)** | $R_1$ = 7.19, $wR_2$ = 22.67 |
| **G.o.F** | 1.014 |
| **CCDC** | 2258898 |

### *4.2 Crystal Structure of Compound 1a*

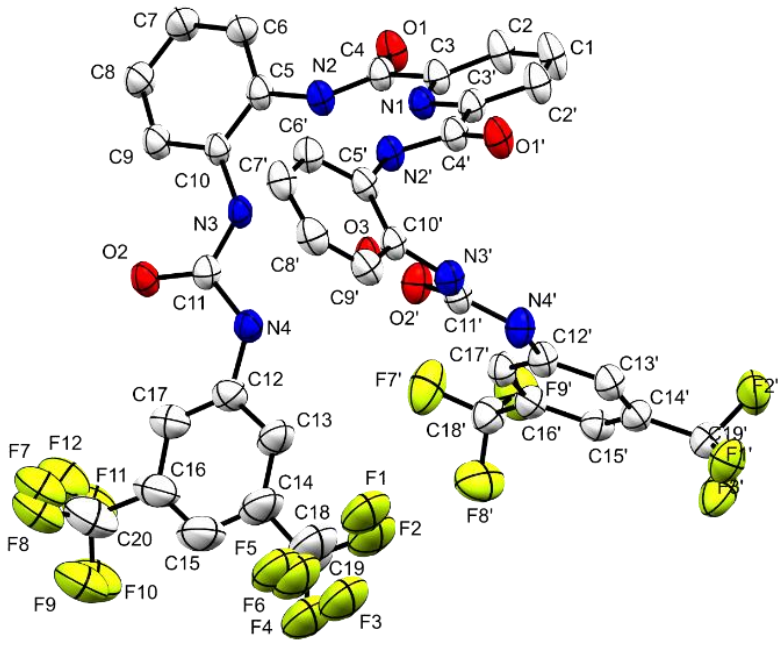



**Fig. S15** ORTEP diagram of Compound **1a**⊃$H_2O$ at 50% probability (H-atoms were omitted for clarity).

## 5. Theoretical Studies

The chloride bound structure of Compound **1a** was optimized using Gaussian 09 software using the following parameters.

Basis set = B3LYP

Level of theory = 6-311g(d,p)

Charge = -1

Multiplicity = 1

| **Symbol** | **X** | **Y** | **Z** |
|---|---|---|---|
| F1 | 3.586183 | -2.625942 | -4.099043 |
| O2 | -4.251461 | 1.89968 | -1.776173 |
| O3 | -4.866737 | -5.004729 | -0.948993 |
| O4 | 3.166418 | 0.184455 | 2.339956 |
| O5 | -1.699949 | -2.574737 | 4.793682 |
| N6 | -3.5183 | -0.281616 | -1.672299 |
| H7 | -2.692432 | -0.853421 | -1.4891 |
| F8 | 5.033336 | -4.05555 | -3.343403 |
| F9 | 7.518304 | -0.74924 | 1.475036 |
| N10 | -3.043024 | -3.313701 | 1.602881 |
| F11 | 5.71467 | -2.270992 | -4.368932 |
| N12 | 0.942671 | -0.326018 | 1.984925 |
| H13 | 0.336311 | -0.828115 | 1.338477 |
| F14 | 8.486258 | -1.174441 | -0.424166 |
| N15 | -4.30147 | -2.780334 | -0.771272 |
| H16 | -3.764977 | -2.160198 | -0.17439 |
| N17 | -1.53471 | -1.317651 | 2.884295 |
| H18 | -1.660643 | -1.356683 | 1.87683 |
| N19 | -2.11197 | 1.425297 | -1.058002 |
| H20 | -1.463677 | 0.650155 | -0.900182 |
| F21 | 7.625356 | 0.782994 | -0.054889 |
| N22 | 2.496542 | -0.910565 | 0.418749 |
| H23 | 1.663879 | -1.212304 | -0.088239 |
| C24 | -3.375163 | 1.088985 | -1.527578 |
| C25 | -4.981319 | -2.214795 | -1.870348 |
| C26 | -4.313444 | -4.081689 | -0.366682 |
| C27 | -3.590486 | -4.338196 | 0.938356 |
| C28 | -4.619044 | -0.91467 | -2.292821 |
| C29 | 0.321709 | 0.297776 | 3.077701 |
| C30 | -1.868539 | -2.435646 | 3.592541 |

| | | | |
|---|---|---|---|
| C31 | -0.939295 | -0.175671 | 3.500027 |
| C32 | 2.282554 | -0.298837 | 1.654907 |
| C33 | -2.46912 | -3.565627 | 2.783211 |
| C34 | 0.890353 | 1.395814 | 3.738437 |
| H35 | 1.856733 | 1.752089 | 3.416993 |
| F36 | 2.524172 | 4.361707 | -0.830927 |
| C37 | 4.840985 | -1.999586 | -2.182357 |
| C38 | -5.334388 | -0.307845 | -3.328841 |
| H39 | -5.06755 | 0.695854 | -3.623401 |
| C40 | -2.416018 | -4.844608 | 3.343772 |
| H41 | -1.955577 | -4.963474 | 4.314926 |
| C42 | 3.710218 | -1.121491 | -0.22429 |
| C43 | 4.955255 | -0.747403 | 0.303709 |
| H44 | 4.994415 | -0.261936 | 1.2663 |
| C45 | -6.018742 | -2.887032 | -2.530211 |
| H46 | -6.262883 | -3.89102 | -2.221795 |
| C47 | 6.116109 | -1.007103 | -0.42194 |
| C48 | 6.08222 | -1.629968 | -1.664308 |
| H49 | 6.991665 | -1.82042 | -2.215504 |
| C50 | -6.379207 | -0.975452 | -3.955919 |
| H51 | -6.919975 | -0.486549 | -4.758512 |
| C52 | 3.669845 | -1.753674 | -1.480979 |
| H53 | 2.714035 | -2.042623 | -1.901645 |
| C54 | 0.227698 | 1.996062 | 4.801245 |
| H55 | 0.688583 | 2.841535 | 5.300089 |
| C56 | 4.787028 | -2.730164 | -3.49522 |
| C57 | -1.616302 | 2.701764 | -0.810926 |
| C58 | -6.711412 | -2.268532 | -3.563801 |
| H59 | -7.51538 | -2.803235 | -4.057351 |
| C60 | -1.593441 | 0.449734 | 4.560969 |
| H61 | -2.553809 | 0.062066 | 4.874373 |
| C62 | -1.016761 | 1.529194 | 5.217777 |
| H63 | -1.535533 | 1.999989 | 6.044796 |
| C64 | -2.960554 | -5.905592 | 2.633903 |
| H65 | -2.922364 | -6.913495 | 3.031958 |
| C66 | -3.563646 | -5.651641 | 1.408629 |
| H67 | -4.022646 | -6.424751 | 0.808019 |
| F68 | -2.453646 | 6.864765 | -2.100477 |
| C69 | 7.430867 | -0.542781 | 0.143343 |
| C70 | -2.373094 | 3.877811 | -0.938354 |
| H71 | -3.405209 | 3.810883 | -1.24256 |
| C72 | -0.275215 | 2.801866 | -0.401445 |
| H73 | 0.315788 | 1.901281 | -0.288783 |

| | | | |
|---|---|---|---|
| F74 | -3.910977 | 6.168078 | -0.653047 |
| C75 | 0.287733 | 4.040839 | -0.12823 |
| F76 | 2.199134 | 3.009447 | 0.834113 |
| C77 | -1.784131 | 5.107591 | -0.657366 |
| F78 | -2.2059 | 7.34823 | -0.000558 |
| F79 | 1.972933 | 5.154896 | 1.110897 |
| C80 | -2.590411 | 6.363116 | -0.847103 |
| C81 | -0.457331 | 5.211697 | -0.252431 |
| H82 | -0.020101 | 6.172419 | -0.02126 |
| C83 | 1.740119 | 4.135273 | 0.25184 |
| Cl84 | -0.616186 | -1.429174 | -0.602325 |

| Item | Value | Threshold | Converged? |
|---|---|---|---|
| Maximum Force | 0.000009 | 0.00045 | YES |
| RMS Force | 0.000001 | 0.0003 | YES |
| Maximum Displacement | 0.000855 | 0.0018 | YES |
| RMS Displacement | 0.000183 | 0.0012 | YES |

## 6. Ion-Transport Studies

### *6.1 Ion-Transport Activity Studies Across EYPC-LUVs⊃HPTS*

#### 6.1.1 Preparation of HEPES Buffer

The HEPES buffer was prepared by mixing 10 mM HEPES and 100 mM NaCl in deionized autoclaved water. The pH of the solution was maintained at 7.0 by adding NaOH (0.5 M) solution to it dropwise.

#### 6.1.2 Preparation of Vesicles

Synthetic Large Unilamellar Vesicles (LUVs) entrapped with HPTS dye were prepared using egg yolk phosphatidylcholine (EYPC) lipids. First, 200 µL of EYPC (25 mg/mL) lipid in chloroform was taken in a clean and dry glass vial. A transparent thin film of the lipid was prepared by purging $N_2$ gas with continuously rotating the glass vial, and then the film was dried under vacuum for 5-6 hr to remove the solvent completely. After that, the thin film was hydrated with 200µL HEPES buffer (1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0). It was then vortex at room temperature for 1 hour with 10 minute intervals and then subjected to freeze-thaw cycle more than 15 times (3 min freeze with liq. $N_2$ and then 3 min thaw at 55 °C). The solution was then extruded 21 times through a 100 nm polycarbonate membrane to get unilamellar vesicles of an average diameter of 100 nm confirmed through DLS experiment (Fig. S31). The unentrapped HPTS dye was then separated from the vesicle through size exclusion chromatography using Sephadex G-50 column and HEPES buffer as eluent. The obtained vesicle solution was then diluted to 1.2 mL with the buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) to get EYPC-LUVs⊃HPTS.

Final condition, inside the vesicle: 1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0, and outside: 10 mM HEPES, 100 mM NaCl, pH = 7.0.

#### 6.1.3 Ion-Transport Experiment Across EYPC-LUVs⊃HPTS

In a clean and dry 3 mL fluorescence cuvette, 1975 µL HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) and 25 µL EYPC-LUVs⊃HPTS were taken, and the cuvette was placed in the spectrofluorometer in a stirring condition. The solution was equilibrated for 2 min before the experiment. The HPTS fluorescence emission intensity was monitored at $\lambda_{em}$ = 510 nm with the excitation at $\lambda_{ex}$ = 450 nm ($t$ = 0 sec). After the start of the experiment, 20 µL of 0.5 M NaOH solution was added at $t$ = 20 sec to generate a pH gradient of 0.8 across the vesicle. Then 20 µL of the transporter solution (required conc.) in DMSO was added at $t$ = 100 sec to

initiate the transport of ions. Finally, the vesicles were lysed after reaching saturation by adding 25 µL of 10% Triton X-100 solution at $t$ = 300 sec, and the experiment was then continued for another 50 sec.

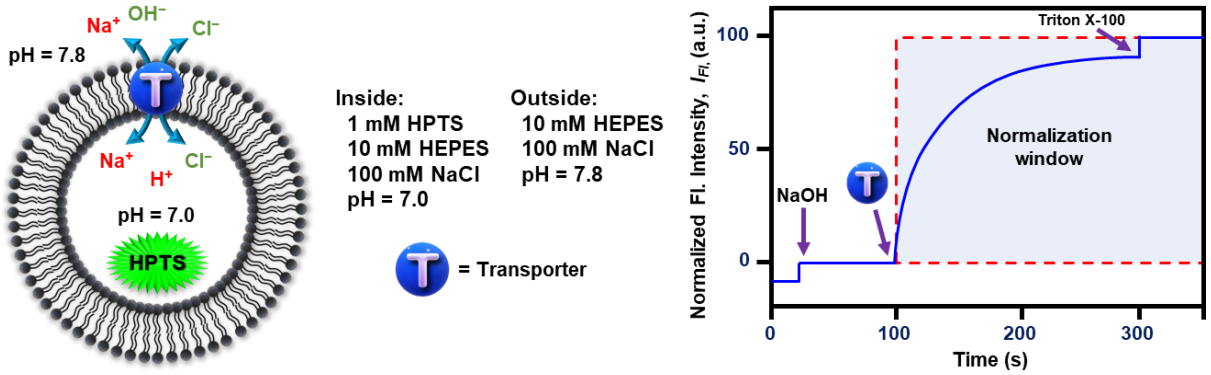



**Fig. S16** Schematic representation of the fluorescence ion-transport assay experiment using EYPC-LUVs⊃HPTS vesicles.

### 6.1.4 Ion-Transport Activity Measurements

The time axis was normalized using the equation; $t = t - 100$.

The fractional fluorescence intensity ($I_F$) was calculated using the following equation;

$$Fractional\ fluorescence\ intensity, I_F = \frac{F_t - F_0}{F_\infty - F_0}$$

where, $F_t$ = Fluorescence intensity at time = $t$

$F_0$ = Fluorescence intensity at time = 0

$F_\infty$ = Fluorescence intensity after the addition of Triton X-100 (300 sec)

### *6.2 Dose-Response Study*

For the dose-response study, the ion-transport activity was monitored by taking the required concentration range of the transporter. Then the normalized fluorescence intensity at $t$ = 295 sec was taken, and the data points at individual concentrations were plotted against the transporter concentration to get the Hill coefficient ($n$) and Effective Concentration ($EC_{50}$) using the following Hill equation;

$$Y = Y_\infty + \frac{(Y_0 - Y_\infty)}{\left[1 + \left(\frac{c}{EC_{50}}\right)\right]}$$

where, $Y_0$ = Fluorescence Intensity at t = 0

$Y_\infty$ = Fluorescence Intensity at t = 200 s

$c$ = Conc. of the transporter molecule

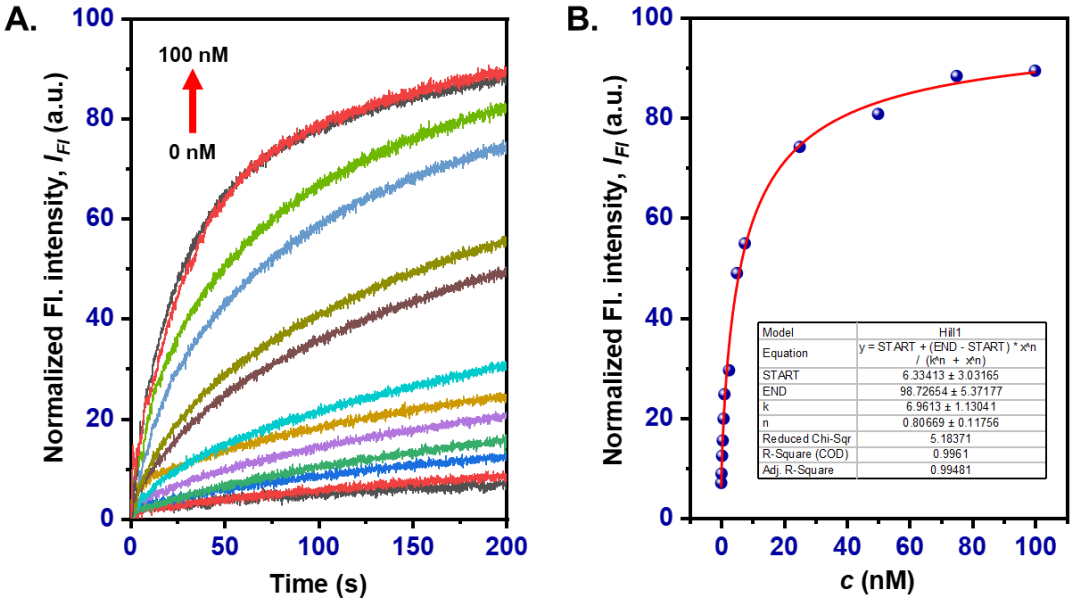



**Fig. S17 (A)** Dose-response ion-transport activity and **(B)** Hill plot of Compound **1a** (0-100 nM) across EYPC-LUVs⊃HPTS.

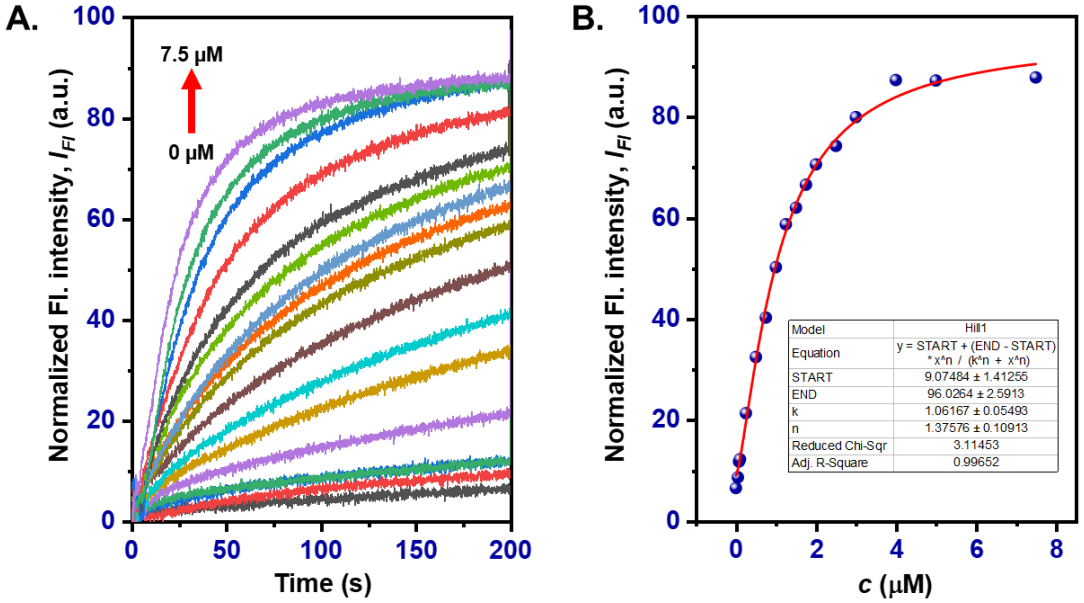



**Fig. S18 (A)** Dose-response ion-transport activity and **(B)** Hill plot of Compound **1b** (0-7.5 μM) across EYPC-LUVs⊃HPTS.

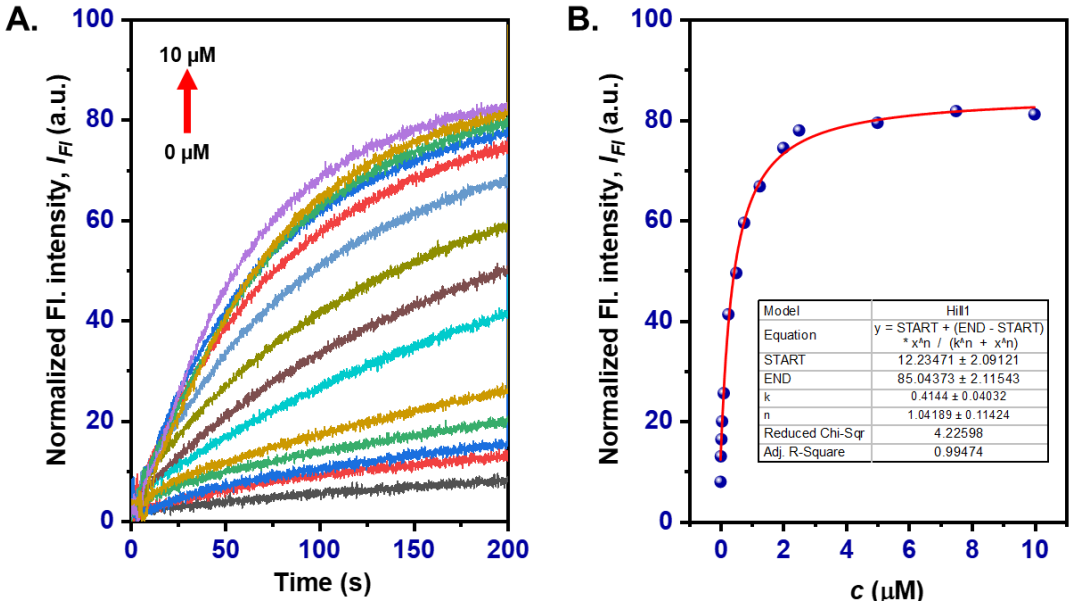



**Fig. S19 (A)** Dose-response ion-transport activity and **(B)** Hill plot of Compound **1c** (0-10 μM) across EYPC-LUVs⊃HPTS.

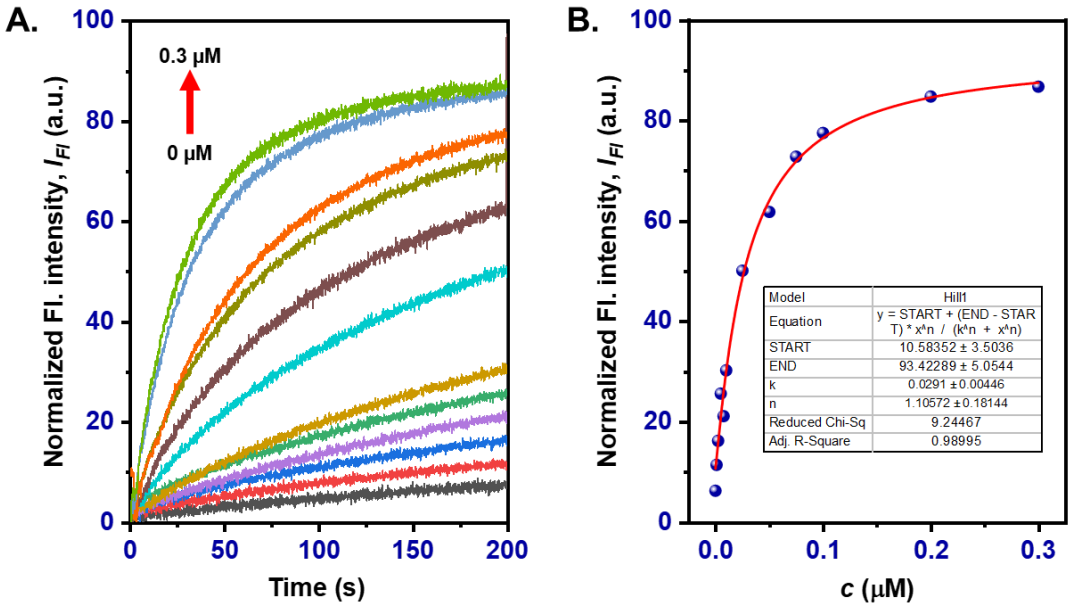



**Fig. S20 (A)** Dose-response ion-transport activity and **(B)** Hill plot of Compound **1d** (0-0.3 μM) across EYPC-LUVs⊃HPTS.

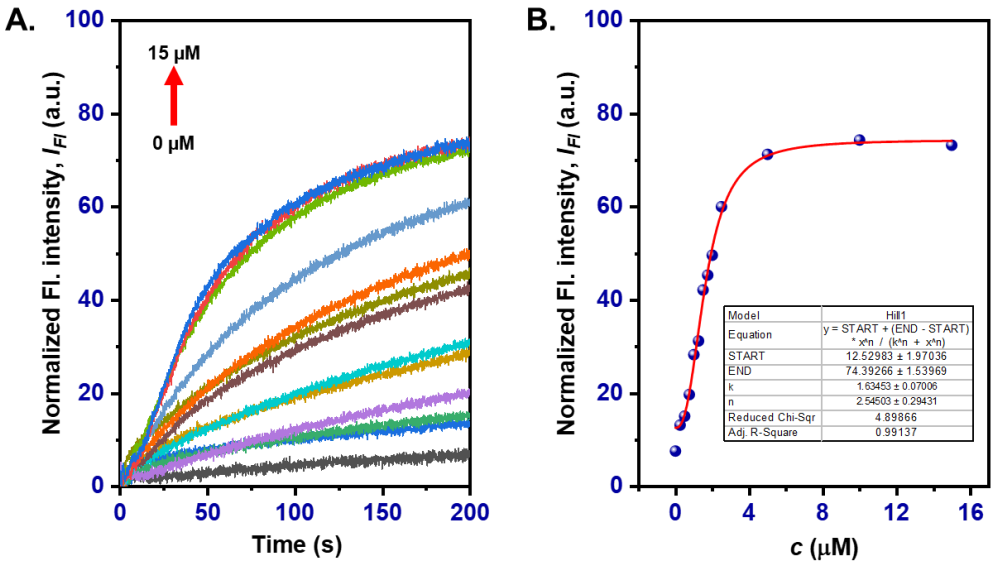



**Fig. S21 (A)** Dose-response ion-transport activity and **(B)** Hill plot of Compound **1e** (0-15 µM) across EYPC-LUVs⊃HPTS.

### *6.3 Ion-Selectivity Study*

#### 6.3.1 Modified HPTS Assay for Anion Selectivity Study

A modified HPTS assay, based on a recently published protocol, was used to examine the anion selectivity of the compounds.[4]

##### *6.3.1.1 HPTS Calibration Experiment*

Large Unilamellar Vesicles (LUVs) for HPTS calibration were prepared as discussed in section 6.1.2, containing 1 mM HPTS, 10 mM HEPES, and 100 mM NaCl buffered to pH = 7.0. Different external buffer solutions with pH ranging from 5.3 to 9.2, containing 10 mM HEPES and 100 mM NaCl, were prepared using the NaOH solution.

For the experiment, 2.5 µL of 0.5 mM monensin solution was added to 1975 µL HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) containing 25 µL of EYPC-LUVs⊃HPTS. The solution was equilibrated for 3 min before the experiment. The HPTS fluorescence emission intensity was monitored at $\lambda_{em}$ = 510 nm with the excitation at two different wavelengths ($\lambda_{ex}$ = 460 nm and 403 nm). The ratiometric fluorescence response of HPTS $I_{460}$ / $I_{403}$ ($\lambda_{ex}$ = 460 nm, $\lambda_{em}$ = 510 nm, divided by $\lambda_{ex}$ = 403 nm, $\lambda_{em}$ = 510 nm) was then converted to pH using the following Henderson-Hasselbalch equation.

$$y = log\frac{ax - b}{c - x}$$

where $y$ = pH

$x = I_{460} / I_{403}$

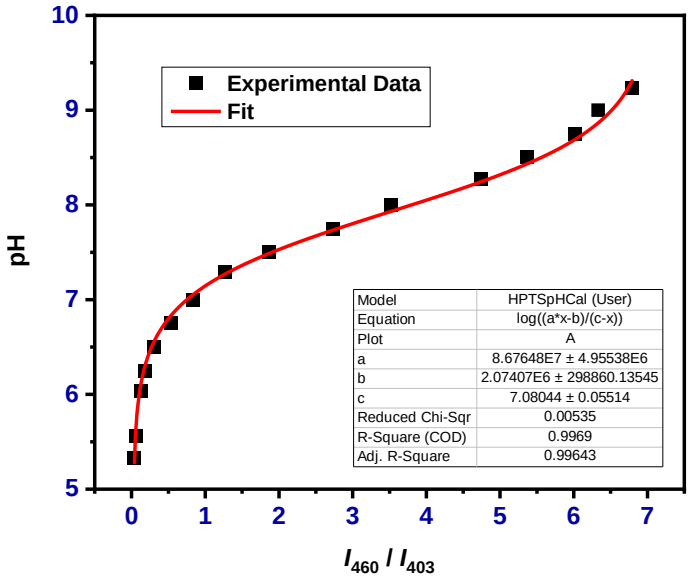



**Fig. S22** Calibration curve for HPTS assay using EYPC-LUVs⊃HPTS vesicles.

### *6.3.1.2 Anion Gradient Assay*

During the anion gradient assay experiment, the external buffer solution was changed to 10 mM HEPES, and 100 mM NaX salts ($X^-$ = $Cl^-$, $Br^-$, $I^-$, $ClO_4^-$, $NO_3^-$ and $AcO^-$), and pH was maintained at 7.0. In a clean and dry 3 mL fluorescence cuvette, 1975 µL HEPES buffer (10 mM HEPES, 100 mM NaX, pH = 7.0) and 25 µL EYPC-LUVs⊃HPTS were taken, and the cuvette was placed in the spectrofluorometer in a stirring condition. The solution was equilibrated for 2 min before the experiment. The HPTS fluorescence emission intensity was monitored at $\lambda_{em}$ = 510 nm with the excitation at $\lambda_{ex}$ = 460 nm ($t$ = 0 sec). After the start of the experiment, 20 µL of Compound **1a** (1 µM stock) was added at $t$ = 50 sec to initiate the transport of ions. The vesicles were then lysed by adding 25 µL of 10% Triton X-100 solution at $t$ = 1050 sec, and the experiment was then continued for another 50 sec. The experiment was repeated at $\lambda_{ex}$ = 460 nm, and the ratiometric fluorescence responses ($I_{460}$ / $I_{403}$) were then converted to $pH_{in}$ using the calibration shown in Fig. S22.

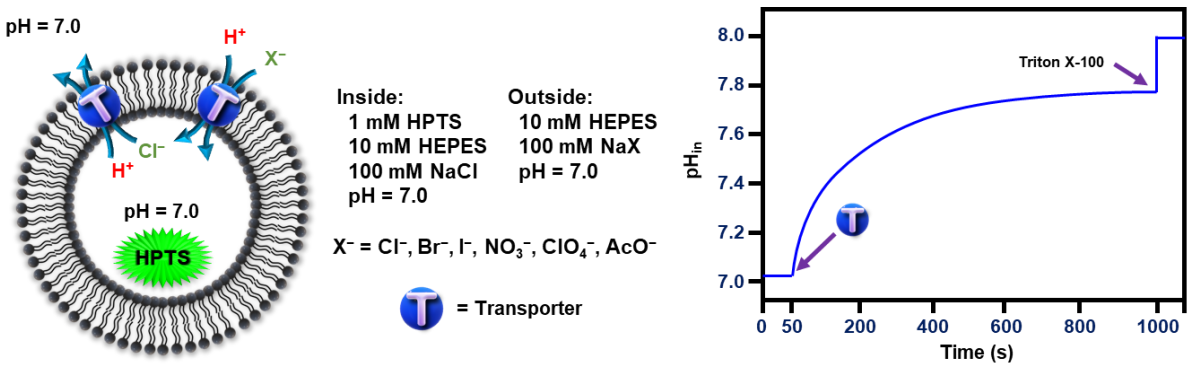



**Fig. S23** Schematic representation of the anion gradient assay experiment using EYPC-LUVs⊃HPTS vesicles.

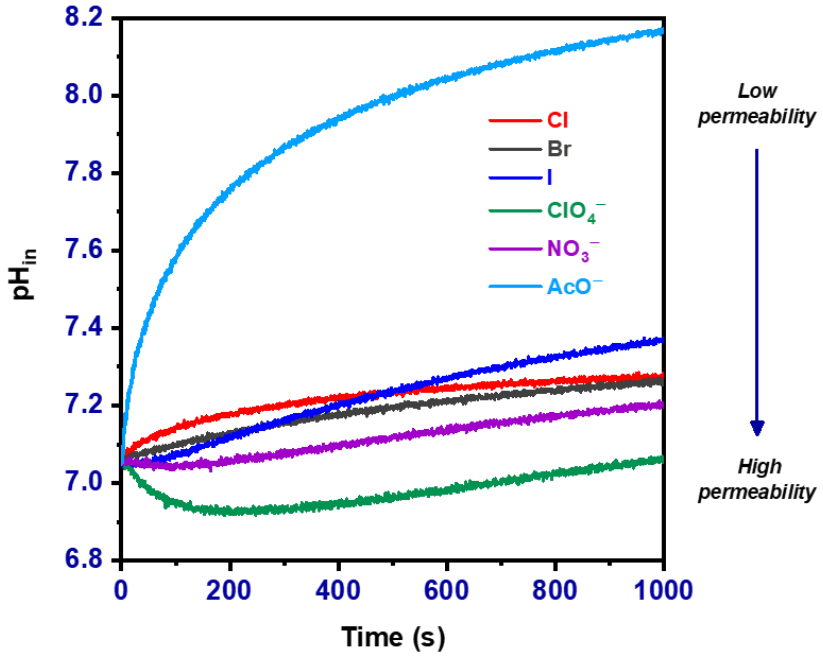



**Fig. S24** The anion gradient assay measured across EYPC-LUVs⊃HPTS vesicles to evaluate the anion transport selectivity of Compound **1a**.

### 6.3.2 Cation Selectivity Study using Dual Gradient Assay

During the cation selectivity assay experiment, the external buffer solution was changed to 10 mM HEPES, and 100 mM MCl salts ($M^+$ = $Li^+$, $Na^+$, $K^+$, $Rb^+$ and $Cs^+$), and pH was maintained at 7.0. In a clean and dry 3 mL fluorescence cuvette, 1975 µL HEPES buffer (10 mM HEPES, 100 mM MCl, pH = 7.0) and 25 µL EYPC-LUVs⊃HPTS were taken, and the cuvette was placed in the spectrofluorometer in a stirring condition. The solution was equilibrated for 2 min before the experiment. The HPTS fluorescence emission intensity was monitored at $\lambda_{em}$ = 510 nm with the excitation at $\lambda_{ex}$ = 450 nm ($t$ = 0 sec). After the start of the experiment, 20 µL of 0.5 M NaOH solution was added at $t$ = 20 sec to generate a pH gradient of 0.8 across the vesicle. Then 20 µL of Compound **1a** (1 µM stock) in DMSO was added at $t$ = 100 sec to initiate the transport of ions. Finally, the vesicles were lysed after reaching saturation by adding 25 µL of 10% Triton X-100 solution at $t$ = 300 sec, and the experiment was then continued for another 50 sec.

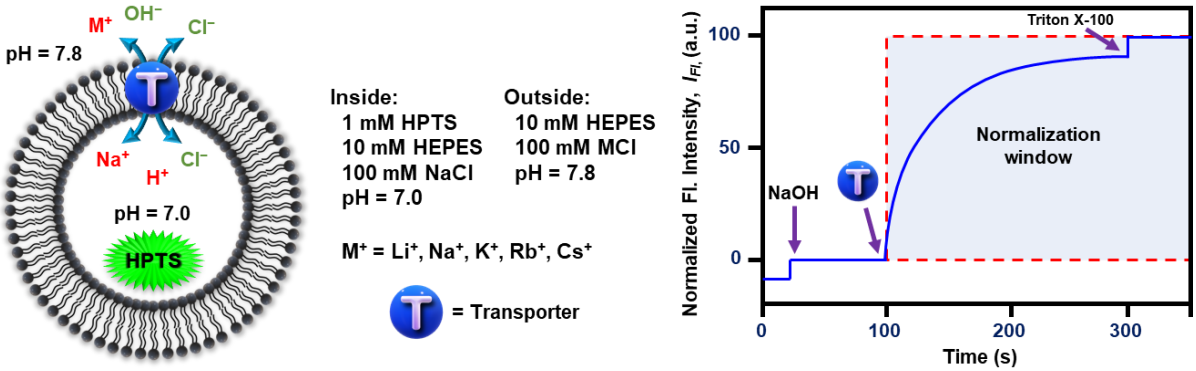



**Fig. S25** Schematic representation of the cation selectivity assay experiment using EYPC-LUVs⊃HPTS vesicles.

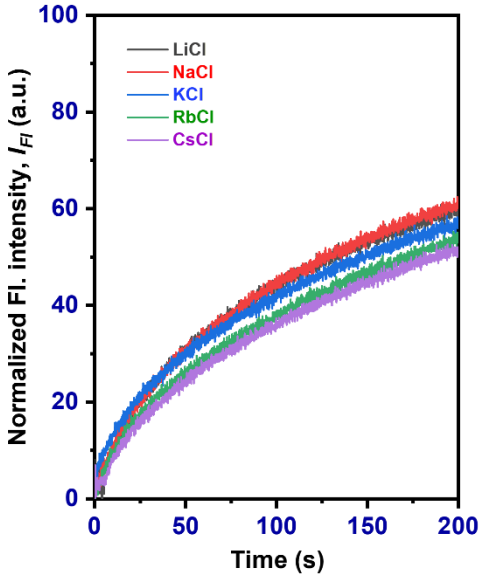



**Fig. S26** Cation selectivity of Compound **1a** (10 nM) measured across EYPC-LUVs⊃HPTS by changing the external buffer.

### *6.4 Mechanistic Study of Ion-transport Across EYPC-LUVs⊃ HPTS*

#### 6.4.1 FCCP Assay Experiment

In a clean and dry 3 mL fluorescence cuvette, 1975 µL HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) and 25 µL EYPC-LUVs⊃HPTS were taken, and the cuvette was placed in the spectrofluorometer in a stirring condition. The solution was equilibrated for 2 min before the experiment. The HPTS fluorescence emission intensity was monitored at $\lambda_{em}$ = 510 nm with the excitation at $\lambda_{ex}$ = 450 nm ($t$ = 0 sec). After the start of the experiment, 20 µL of 0.5 M NaOH solution was added at $t$ = 20 sec to generate a pH gradient of 0.8 across the vesicle. Then 10 µL of FCCP (200 µM stock) and 10 µL of Compound **1a** (1 µM) were added at a time at $t$ = 100 sec to initiate the transport of ions. Finally, the vesicles were lysed after reaching

saturation by adding 25 µL of 10% Triton X-100 solution at *t* = 300 sec, and the experiment was then continued for another 50 sec. For the control experiment, 20 µL of FCCP was added at t = 100 sec instead of Compound **1a**.

### 6.4.2 Valinomycin Assay Experiment

In a clean and dry 3 mL fluorescence cuvette, 1975 µL HEPES buffer (10 mM HEPES, 100 mM KCl, pH = 7.0) and 25 µL EYPC-LUVs⊃HPTS were taken, and the cuvette was placed in the spectrofluorometer in a stirring condition. The solution was equilibrated for 2 min before the experiment. The HPTS fluorescence emission intensity was monitored at $\lambda_{em}$ = 510 nm with the excitation at $\lambda_{ex}$ = 450 nm (*t* = 0 sec). After the start of the experiment, 20 µL of 0.5 M NaOH solution was added at *t* = 20 sec to generate a pH gradient of 0.8 across the vesicle. Then 10 µL of Valinomycin (200 pM stock) and 10 µL of Compound **1a** (1 µM) were added at a time at *t* = 100 sec to initiate the transport of ions. Finally, the vesicles were lysed after reaching saturation by adding 25 µL of 10% Triton X-100 solution at *t* = 300 sec, and the experiment was then continued for another 50 sec. For the control experiment, 20 µL of Valinomycin was added at t = 100 sec instead of Compound **1a**.

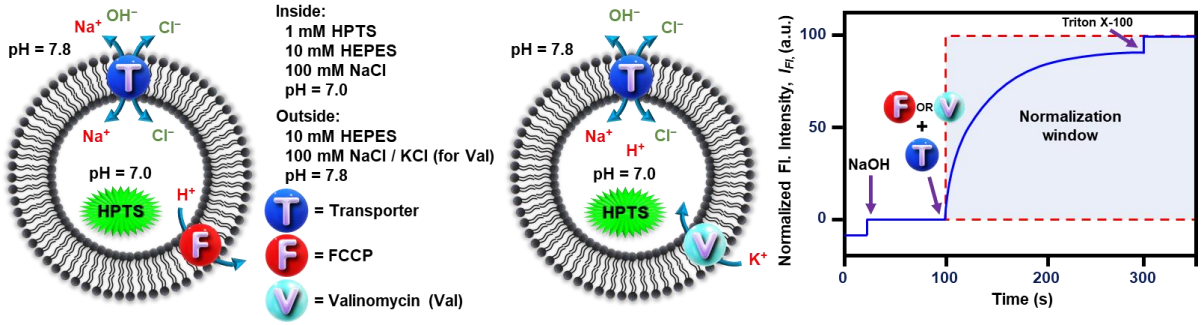



**Fig. S27** Schematic representation of FCCP and Valinomycin assay experiment using EYPC-LUVs⊃HPTS vesicles.

### *6.5 U-tube Experiment*

In the conventional U-tube experiment, the two aqueous phase were separated by a layer of $CHCl_3$ which was used to mimic the lipid bilayer system. The source arm and the receiver arm were filled with 10 mL of 100 mM HCl, and 10 mL of 100 mM $NaNO_3$ respectively which was separated by 0.5 mM of compound **1a** (18 mL) in $CHCl_3$. The organic layer was gently stirred and the pH of the receiver arm was measured for 42 hrs using a pH meter.

### *6.6 Ion-Transport Activity Studies Across EYPC/CHOL-LUVs⊃ Lucigenin*

#### 6.6.1 Preparation of Vesicles

Large Unilamellar Vesicles (LUVs) entrapped with Lucigenin dye were prepared using cholesterol and egg yolk phosphatidylcholine (EYPC) lipids. Cholesterol (1.07 mg) and 200 µL of EYPC (25 mg/mL) lipid in 7:3 molar ratio was taken in chloroform in a clean and dry glass vial. A transparent thin film of the lipid was prepared by purging $N_2$ gas with continuously rotating the glass vial, and then the film was dried under vacuum for 5-6 hr to remove the solvent completely. After that, the thin film was hydrated with 200µL buffer solution (1 mM Lucigenin, 200 mM $NaNO_3$, pH = 7.0). It was then vortex at room temperature for 1 hour with 10-minute intervals and then subjected to freeze-thaw cycles more than 15 times (3 min freeze with liq. $N_2$ and then 3 min thaw at 55 °C). The solution was then extruded 21 times through a 200 nm polycarbonate membrane to get unilamellar vesicles of an average diameter of 200 nm. The unentrapped lucigenin dye was then separated from the vesicle through size exclusion chromatography using Sephadex G-50 column and buffer as eluent. The obtained vesicle solution was then diluted to 0.6 mL with the buffer (200 mM $NaNO_3$, pH = 7.0) to get EYPC/CHOL-LUVs⊃Lucigenin. Final condition, inside the vesicle: 1 mM Lucigenin, 200 mM $NaNO_3$, pH = 7.0, and outside: 200 mM $NaNO_3$, pH = 7.0.

#### 6.6.2 Ion-Transport Experiment Across EYPC/CHOL-LUVs⊃Lucigenin

In a clean and dry 3 mL fluorescence cuvette, 1975 µL $NaNO_3$ solution (200 mM $NaNO_3$, pH = 7.0) and 25 µL EYPC/CHOL-LUVs⊃Lucigenin was taken, and the cuvette was placed in the spectrofluorometer in a stirring condition at 30 °C. The solution was equilibrated for 2 min before the experiment. The lucigenin fluorescence emission intensity was monitored at $\lambda_{em}$ = 535 nm with the excitation at $\lambda_{ex}$ = 450 nm ($t$ = 0 sec). After the start of the experiment, 33.3 µL of 2 M NaCl solution was added at $t$ = 20 sec to generate a chloride gradient across the vesicle. Then 20 µL of Compound **1a** (5 µM stock) in acetone was added at $t$ = 100 sec to initiate the transport of ions. Finally, the vesicles were lysed after reaching saturation by adding 25 µL of 10% Triton X-100 solution at $t$ = 400 sec, and the experiment was then continued for another 50 sec.

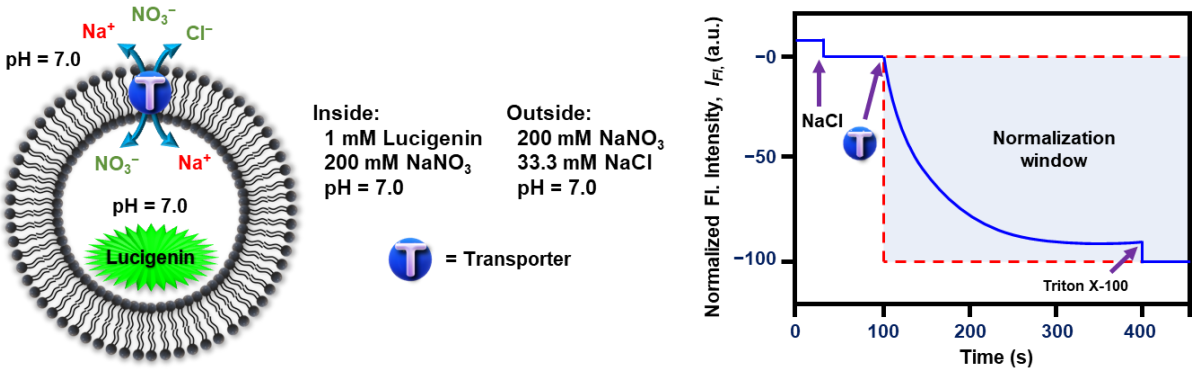



**Fig. S28** Schematic representation of the lucigenin-based fluorescence ion-transport assay experiment using EYPC/CHOL-LUVs⊃Lucigenin vesicles.

### 6.6.3 Ion-Transport Activity Measurements

The time axis was normalized using the equation; $t = t - 100$.

The fractional fluorescence intensity ($I_F$) was calculated using the following equation;

$$Fractional\ fluorescence\ intensity, I_F = \frac{F_t - F_0}{F_\infty - F_0}$$

where, $F_t$ = Fluorescence intensity at time = $t$

$F_0$ = Fluorescence intensity at time = 0

$F_\infty$ = Fluorescence intensity after the addition of Triton X-100 (400 sec)

## *6.7 Physiological Stability Study*

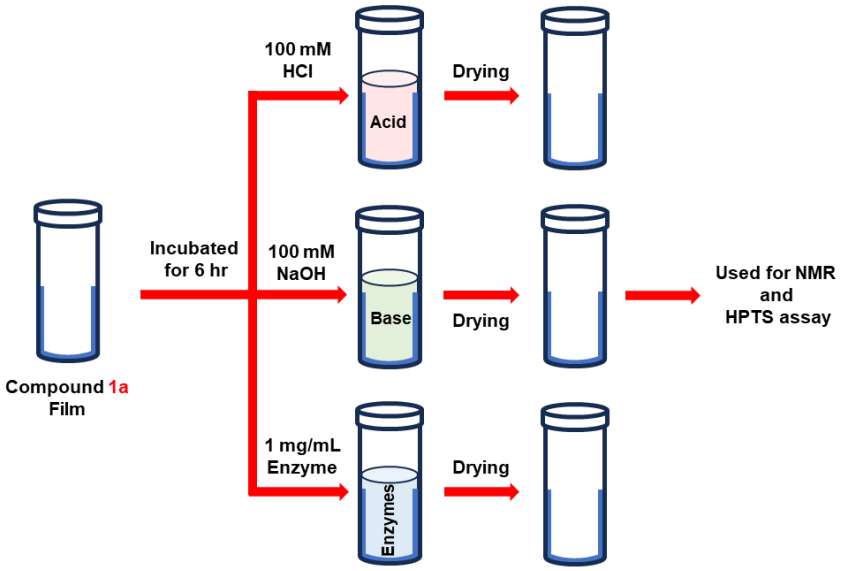



**Fig. S29** Schematic representation of physiological stability study.

We investigated the physiological stability of compound **1a** through $^{1}$H NMR and HPTS assay experiments. For that, films of compound **1a** were made in acetone and dried for 1 hr in vacuo. Then the films were incubated in 100 mM HCl, 100 mM NaOH, and 1 mg/mL of different enzymes such as esterase from porcine liver, lipase, trypsin, and pepsin separately for 6 hrs and then dried in a desiccator for another 5 hrs.

For the study of chemical stability, films incubated in acid and base were then re-dissolved in DMSO-$d_6$ and sent for $^{1}$H NMR experiment.

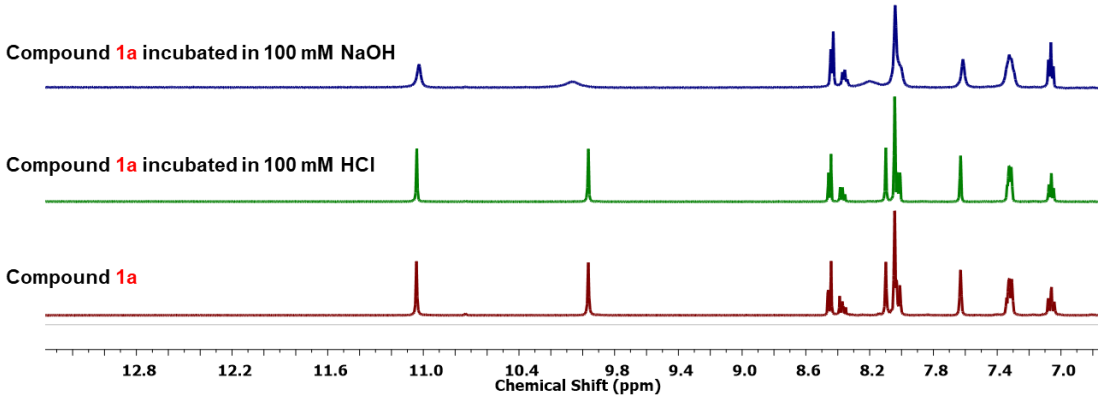



**Fig. S30** Stacked $^{1}$H NMR plot of compound **1a** after incubation in acid and base.

For the HPTS assay experiment, all the films were made as described above and then dissolved in HPLC grade DMSO to prepare the transporter solution of the required concentration and the HPTS assay was conducted as described in section 6.1.3

### *6.8 Variable Temperature HPTS Assay Across DPPC-LUVs⊃HPTS*

#### 6.8.1 Preparation of Vesicles

The vesicle entrapped with HPTS dye was prepared using a similar protocol as discussed in section 6.1.2. But this time, 1,2-dipalmitoyl-*sn*-glycerol-3-phosphocholine (DPPC) lipid (25 mg/mL) was used to prepare the vesicles instead of EYPC lipid.

#### 6.8.2 DPPC Assay Experiment

In a clean and dry 3 mL fluorescence cuvette, 1975 µL HEPES buffer (10 mM HEPES, 100 mM NaCl, pH = 7.0) and 25 µL DPPC-LUVs⊃HPTS were taken, and the cuvette was placed in the spectrofluorometer in a stirring condition. The solution was equilibrated for 3 min at 25 °C before the experiment. The HPTS fluorescence emission intensity was monitored at $\lambda_{em}$ = 510 nm with the excitation at $\lambda_{ex}$ = 450 nm ($t$ = 0 sec). After the start of the experiment, 20 µL of 0.5 M NaOH solution was added at $t$ = 20 sec to generate a pH gradient of 0.8 across

the vesicle. Then 20 µL of Compound **1a** (2.5 µM stock) in DMSO was added at *t* = 100 sec to initiate the transport of ions. Finally, the vesicles were lysed after reaching saturation by adding 25 µL of 10% Triton X-100 solution at *t* = 300 sec, and the experiment was then continued for another 50 sec.

The same experiment was conducted at 45 °C (above the gel-to-liquid crystal transition temperature of DPPC lipid). A substantial increment in the ion transport activity at a higher temperature (45 °C) provides the evidence of carrier-mediated transport than a channel. Similarly, the control experiments were conducted without the transporter (20 µL DMSO only) at both 25 and 45 °C temperature which shows no transport of ions by the transporter.

### *6.9 DLS Experiment*

For the DLS experiment, 25 µL vesicle solution and 975 µL HEPES buffer (10 mM HEPES, 100 mM NaCl) were mixed in a 1 mL cuvette, and the mean particle size was measured using Delsa Nano (Beckman Coulter) instrument. The average diameter of the particle is 115.6 nm, and the polydispersity index (PDI) is 0.09.

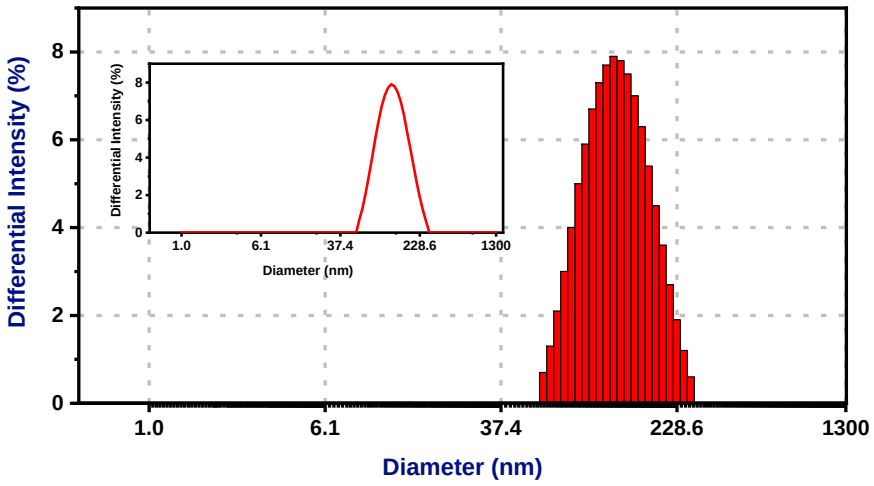



**Fig. S31** DLS plot of EYPC-LUVs⊃HPTS vesicles in HEPES buffer solution.

## 7. Cellular Studies

### *7.1 MTT-based cytotoxicity Assay*

For cell viability experiments, cancerous HeLa and non-cancerous HEK-293T were procured from ATCC. Both the cell lines were cultured at 37 °C and 5% $CO_2$ in Dulbecco's Modified Eagle's Medium (DMEM, purchased from HiMedia Laboratories Pvt. Ltd., lot no.: 0000437528), 10% fetal bovine serum (procured from HiMedia Laboratories Pvt. Ltd., lot no.:

0000416227) and 1% penicillin-streptomycin antibiotic solution (purchased from Thermo Fisher Scientific, lot no.: 1894163)

HeLa and HEK-293T cells were seeded in a 96-well plate at a density of 5000 cells/well and were allowed to adhere to the surface for 24 hr prior to the treatment of the compound. After 24 hr, the media was discarded, and the plate was washed with PBS buffer. Then 1% DMSO solution of the compound was added with media in different conc. and incubated for 24 hr at 37 °C and 5% $CO_2$. After incubation, the media was removed and replaced with media containing 5 mg MTT/mL and incubated for 4 hr. The MTT-containing solution was discarded carefully, and the formazan crystals were dissolved in 200 µL DMSO. The absorbance was taken at 570 nm after 30 min using a microplate reader (Synergy-HT, Bio-Tech Instruments, Inc.). All the experiments were carried out in triplicate, and the relative cell viability (%) was plotted in comparison to the untreated cells. The $IC_{50}$ calculation was done from MTT assay using Probit analysis method which comes out to be 19.84 µM.

## 8. NMR Spectra

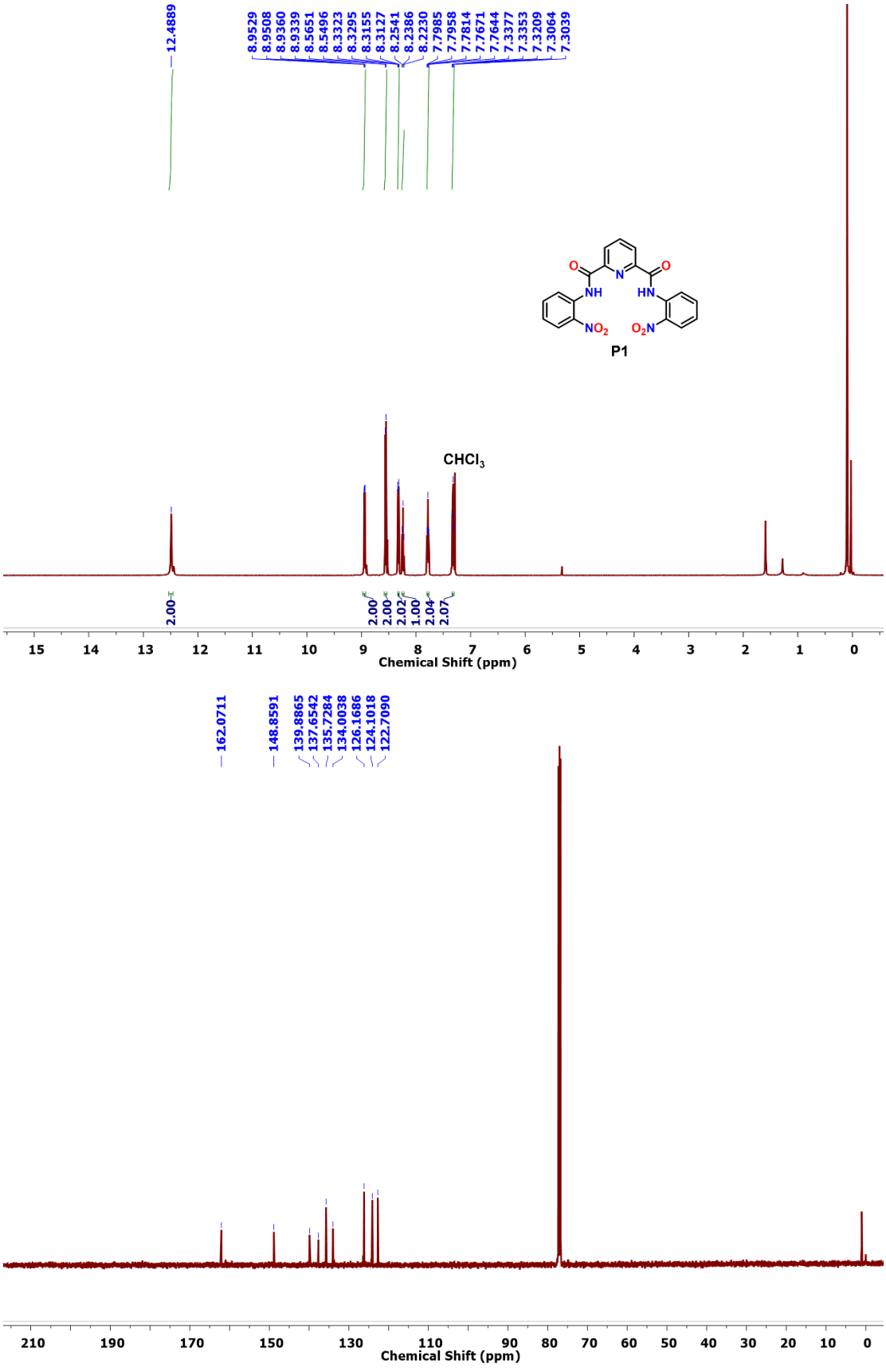



**Fig. S32** $^{1}H$ and $^{13}C$ NMR spectra of precursor **P1** in $CDCl_3$ (500 MHz, 25 °C).

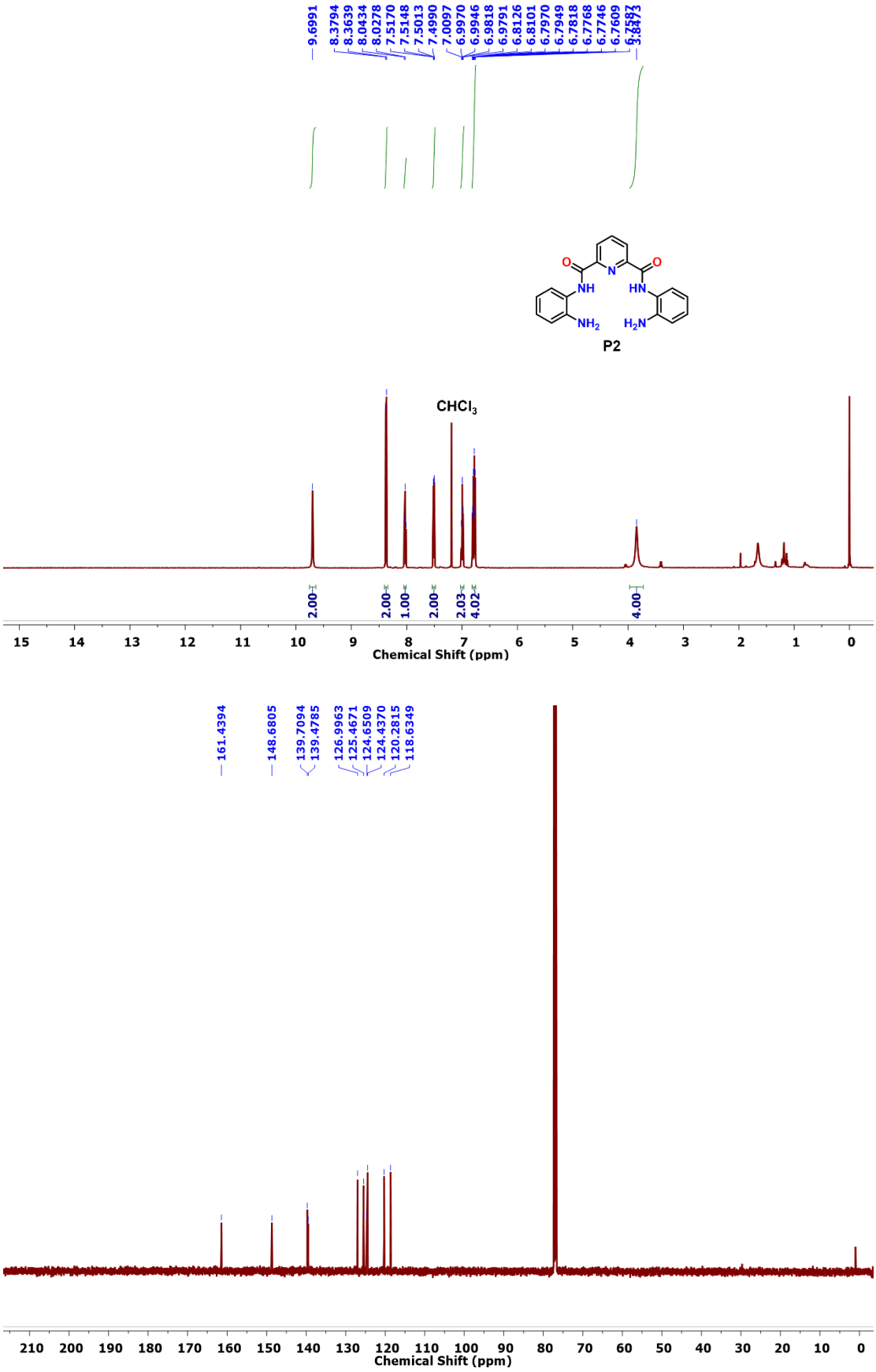



**Fig. S33** $^{1}H$ and $^{13}C$ NMR spectra of precursor **P2** in $CDCl_3$ (500 MHz, 25 °C).

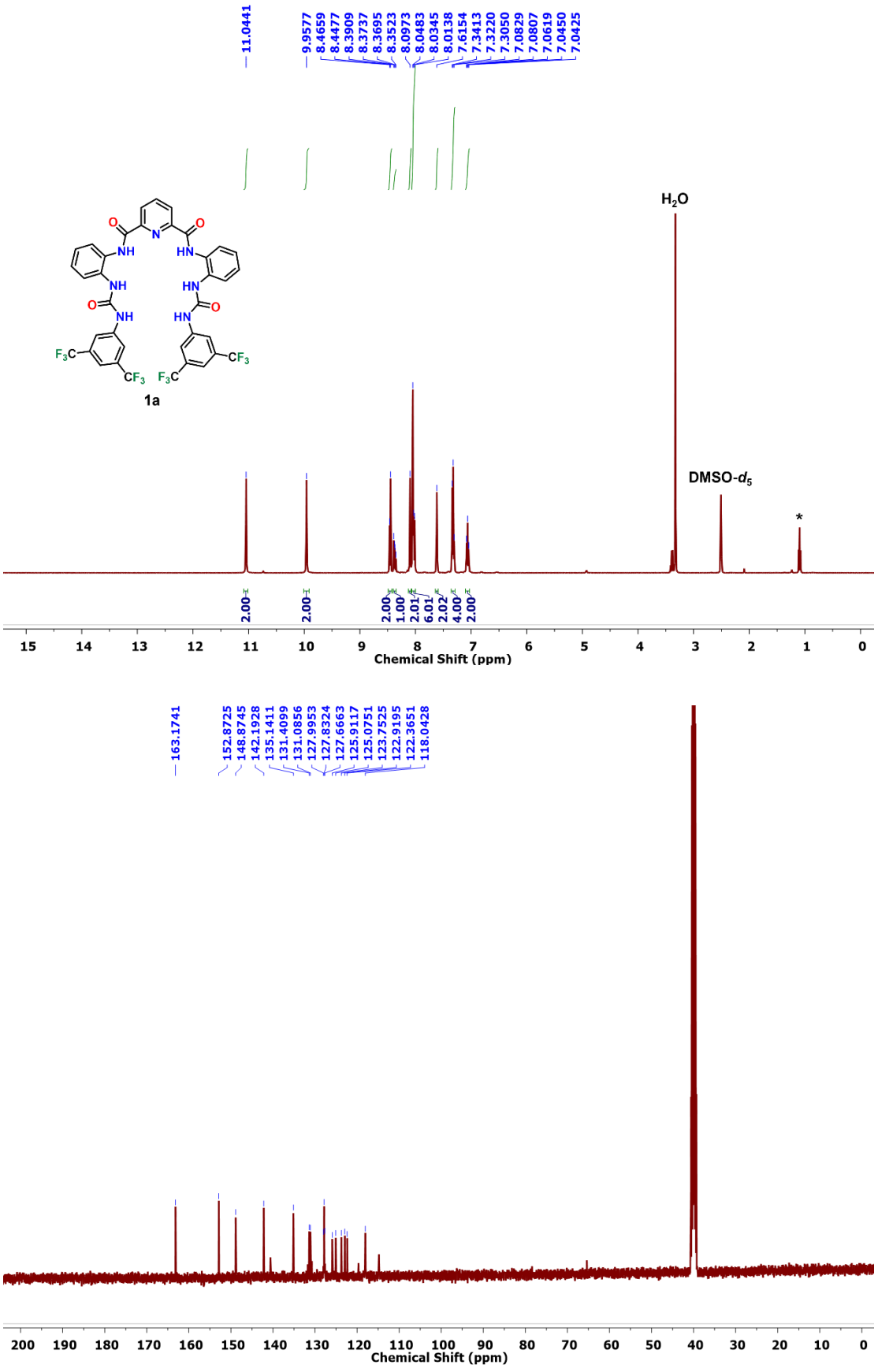



**Fig. S34** $^{1}$H and $^{13}$C NMR spectra of Compound **1a** in DMSO-$d_6$ (500 MHz, 25 °C).

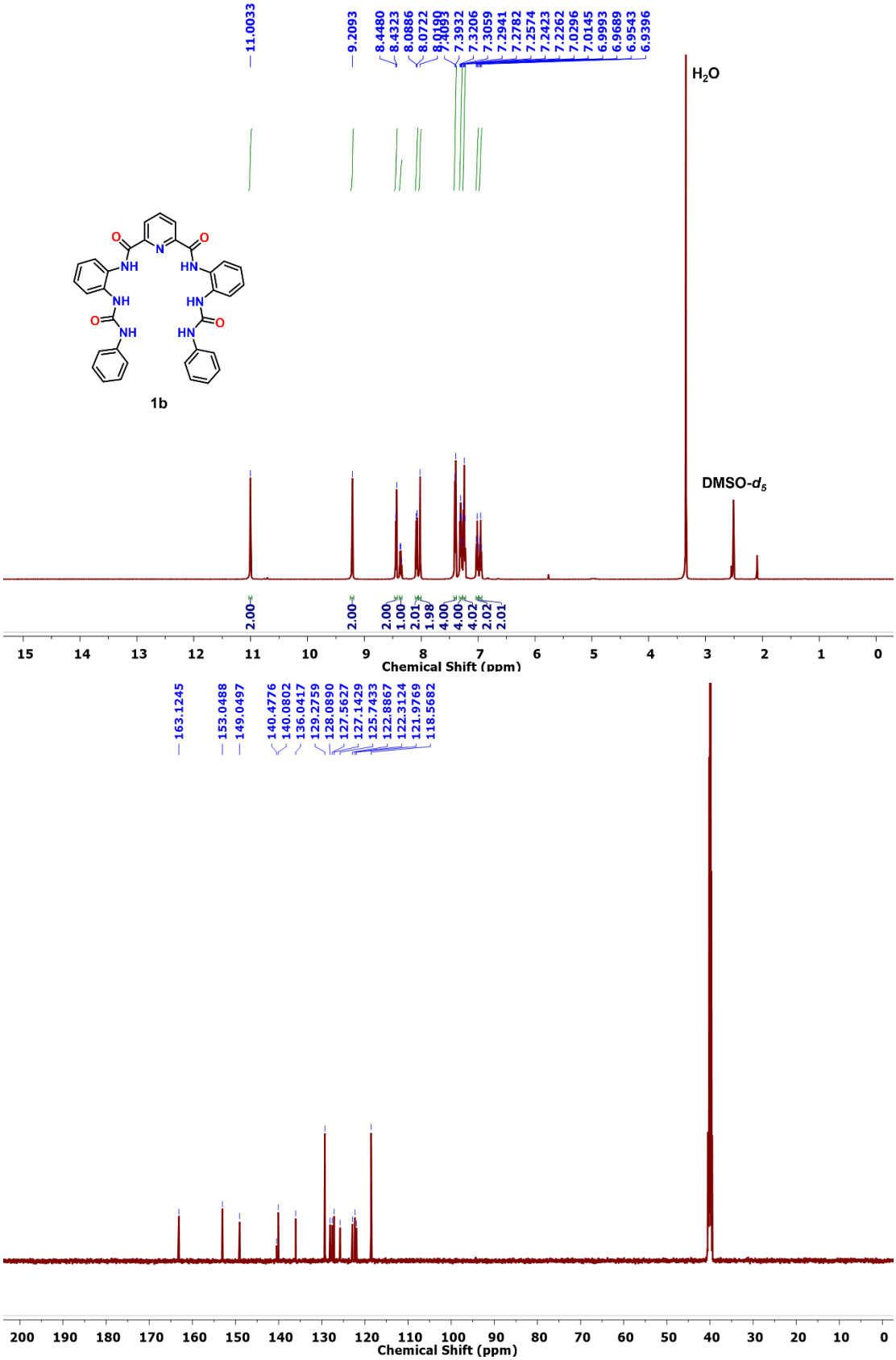



**Fig. S35** $^{1}$H and $^{13}$C NMR spectra of Compound **1b** in DMSO-$d_6$ (500 MHz, 25 °C).

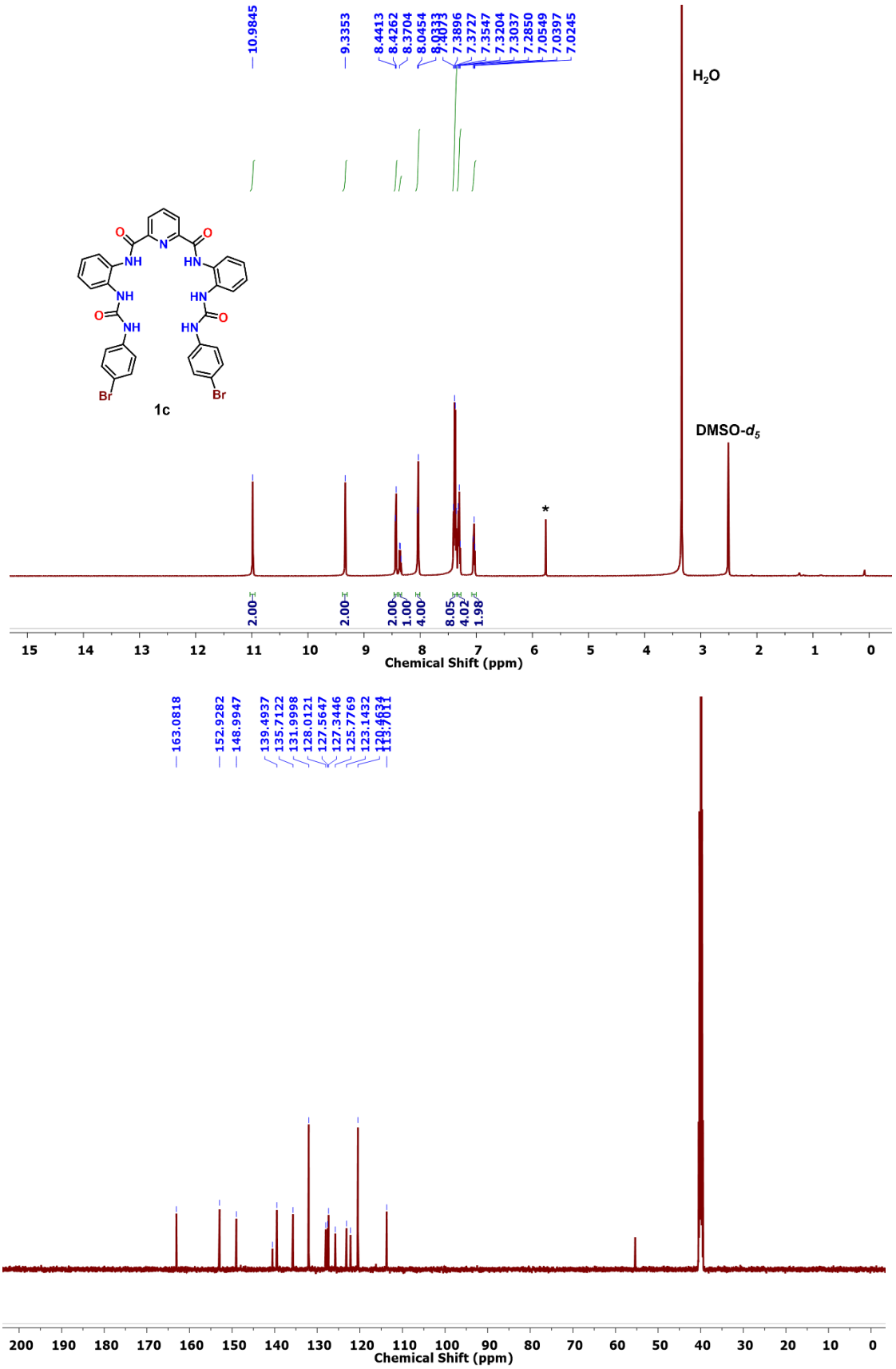



**Fig. S36** $^{1}$H and $^{13}$C NMR spectra of Compound **1c** in DMSO-$d_6$ (500 MHz, 25 °C).

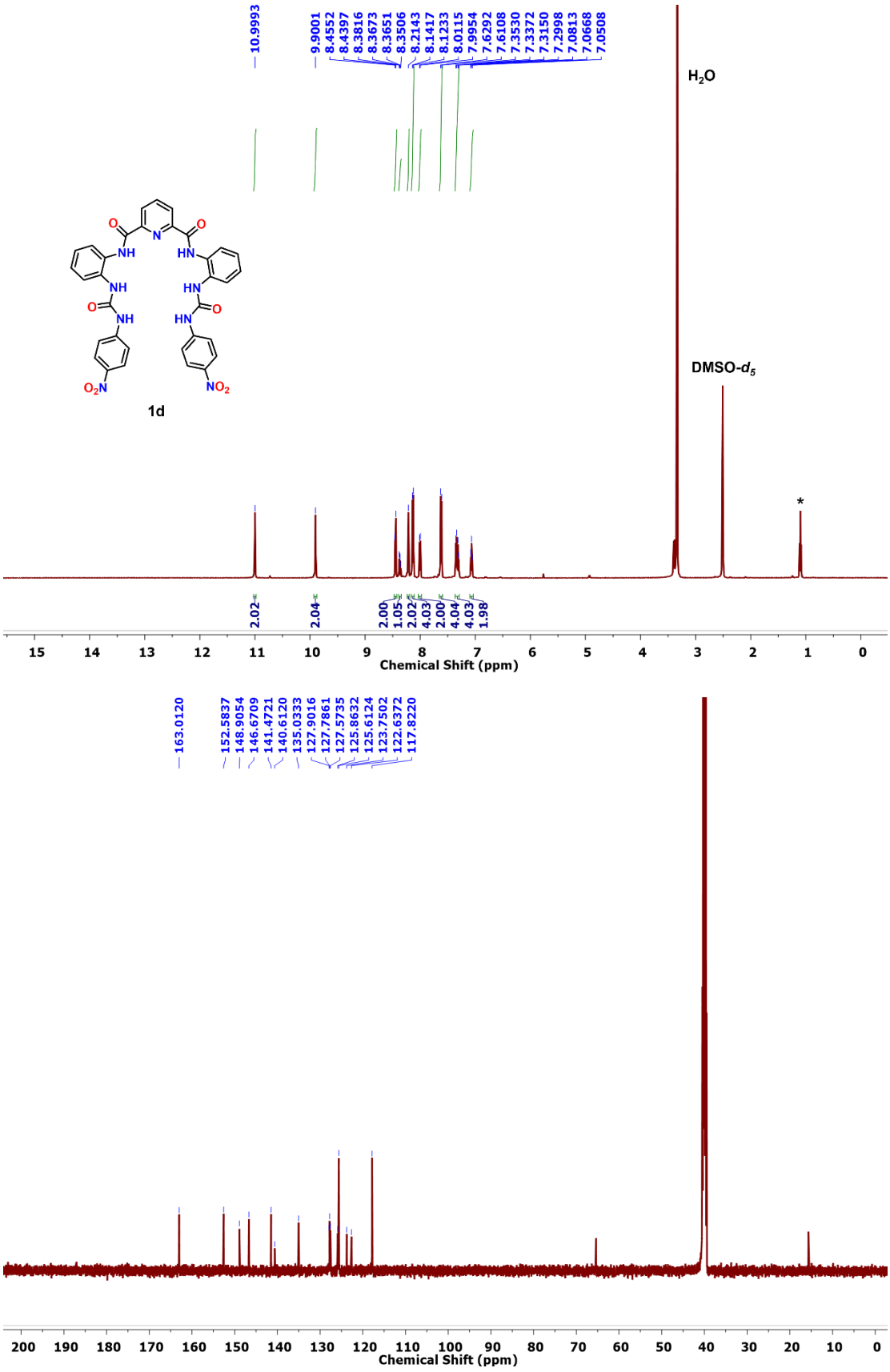



**Fig. S37** $^{1}$H and $^{13}$C NMR spectra of Compound **1d** in DMSO-$d_6$ (500 MHz, 25 °C).

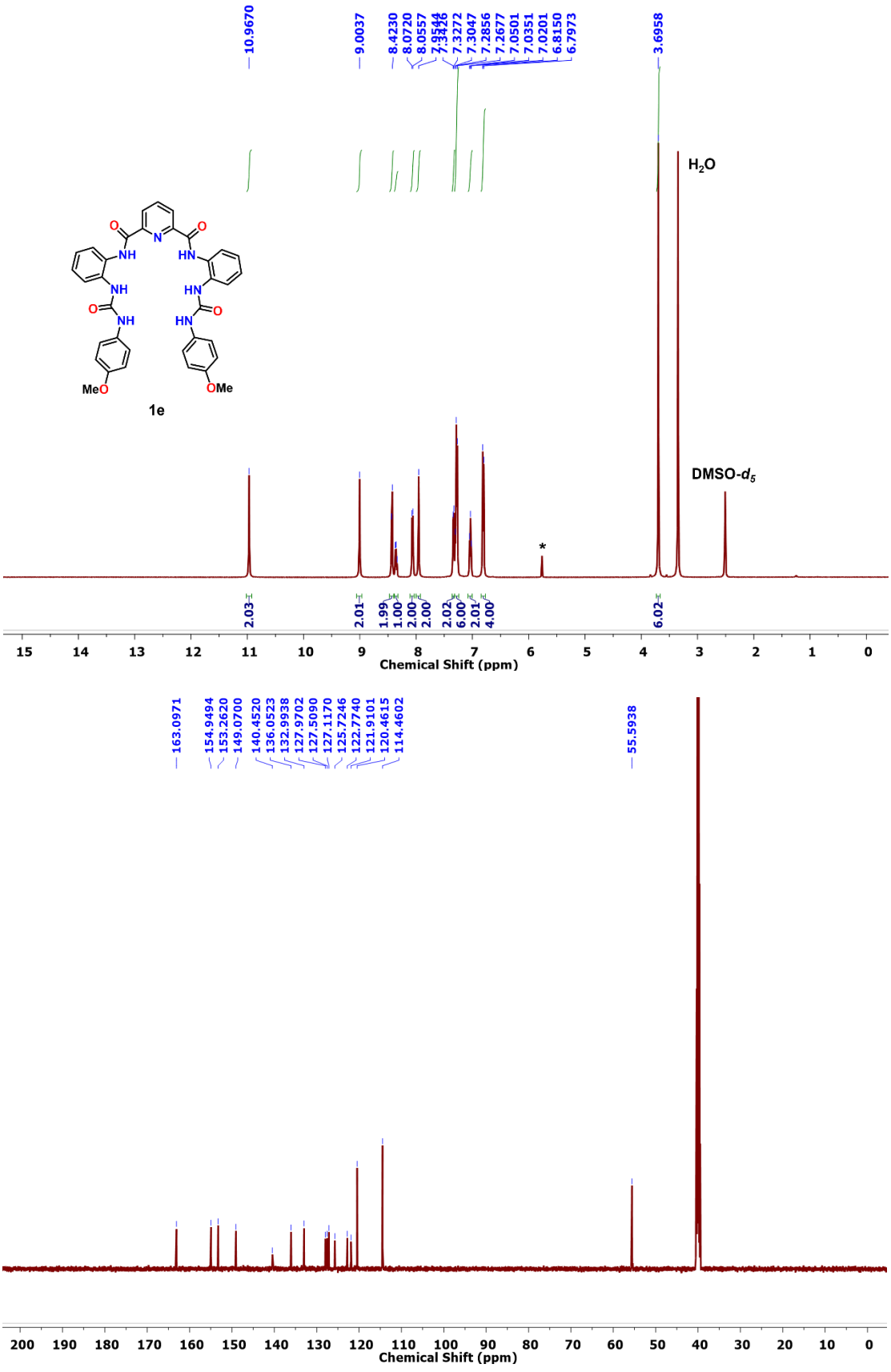



**Fig. S38** $^{1}$H and $^{13}$C NMR spectra of Compound **1e** in DMSO-$d_6$ (500 MHz, 25 °C).

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