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Supporting Information

Bis(sulfonamide) transmembrane carrier allows pH-gated inversion of ion selectivity

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I. General Methods

All reactions were carried out under the nitrogen atmosphere. All the chemicals were purchased from commercial sources and were used as received unless stated otherwise. Solvents were dried by standard methods prior to use or purchased as dry. Thin layer chromatography (TLC) was carried out with E. Merck silica gel 60-F_{254} plates and column chromatography was performed over silica gel (100-200 mesh) obtained from commercial suppliers. Egg yolk phosphatidylcholine (EYPC) lipid was purchased from Avanti Polar Lipids as a solution dissolved in chloroform (25 mg/mL). HEPES buffer, monobasic sodium phosphate salt, dibasic sodium phosphate salt, HPTS dye, Safranin O dye, Triton X-100, NaOH and all inorganic salts of molecular biology grade were purchased from Sigma. Gel-permeation chromatography was performed on a column of Sephadex LH-20 gel (25×300 mm, V_0 = 25 mL). Large unilamellar vesicles (LUV) were prepared from EYPC lipid by using mini extruder, equipped with a polycarbonate membrane either of 100 nm or 200 nm pore size, obtained from Avanti Polar Lipids.

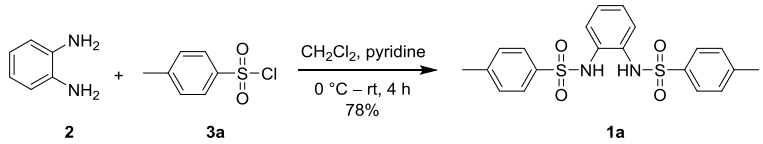
II. Physical Measurements

The ^{1}H and ^{13}C NMR spectra were recorded on 400 MHz Jeol ECS-400 (or 100 MHz for ^{13}C) spectrometers using either residual solvent signals as an internal reference or from internal tetramethylsilane on the δ scale relative to chloroform (δ 7.26), dimethylsulphoxide (δ 2.50 ppm), acetone (δ 2.05) for ^{1}H NMR and chloroform (δ 77.20 ppm), dimethylsulphoxide (δ 39.50 ppm), acetone (δ 29.84 and 206.26) for ^{13}C NMR. The chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The following abbreviations are used: s (singlet), d (doublet) m (multiplet), td (triplet of doublet) while describing ^{1}H NMR signals. High-resolution mass spectra (HRMS) were obtained from MicroMass ESI-TOF MS spectrometer. Fluorescence spectra were recorded by using Fluoromax-4 from Jobin Yvon Edison equipped with an injector port and a magnetic stirrer. 10 mM HEPES (with 100 mM NaCl or other salts as per necessity) buffer solutions were used for fluorescence experiment and the pH of the buffers were adjusted to 7.0 or 8.0 by NaOH and pH of the buffer solutions were measured using Helmer pH meter. 5 mM sodium phosphate buffer was prepared by mixing proper amount of monobasic and dibasic sodium phosphate solution and further pH was adjusted with extra monobasic or dibasic sodium

phosphate solution with NaCl or $NaNO_3$ (500 mM) as per requirement and pH of the buffer solution was measured using Helmer pH meter for chloride ion efflux assays by Chloride Ion Selective Electrode (ISE). Chloride efflux was monitored by Accumet Chloride Combination Ion Selective Electrode furnished with Fisher AB250 pH/Ion meter, obtained from Fischer Scientific. (FT-IR) spectra were obtained using NICOLET 6700 FT-IR spectrophotometer as KBr disc and reported in cm^{-1}. Melting point of all compounds were measured using a VEEGO Melting point apparatus. All melting points were measured in open glass capillary and values are uncorrected. All fluorescence data were processed either by Origin 8.5 or KaleidaGraph and Chloride ISE data were processed by Origin 8.5 and finally all data were processed through ChemDraw Professional 15.

III. Synthesis:

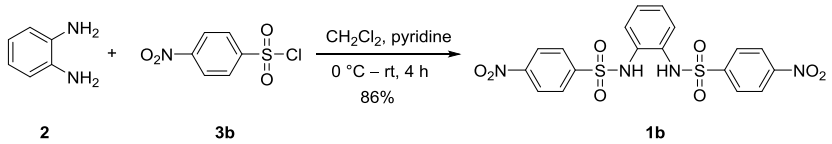
Synthesis of *N, N′*-(1, 2-phenylene)bis(4-methylbenzenesulfonamide) (1a):[S1]



Scheme S1. Synthesis of compound **1a**.

In a 50 mL round bottom flask, *o*-phenylenediamine **2** (500 mg, 4.62 mmol) was dissolved in 10 mL of dry CH_2Cl_2 and followed by 0.5 mL of pyridine was added and reaction mixture was cooled to 0 ^oC. Subsequently, tosyl chloride **3a** (1.89 g, 9.25 mmol) dissolved in 5 mL dry CH_2Cl_2 was added dropwise to the reaction mixture and was stirred for 30 min at 0 ^oC in stirring condition. Then the temperature was brought to room temperature and the reaction mixture was stirred for 4 h and TLC was monitored till the completion of reaction. The reaction mixture was then concentrated to remove CH_2Cl_2 and pyridine and column chromatography was performed using ethyl acetate/pet ether solvent system to obtain 1.5 g of crystalline solid, white product **1a** (78%). **Eluent**: 22% EtOAc/PE. **mp:** 207 ^0C; **IR (KBr):** ν/cm^{-1}: 3317, 3220, 1917, 1805, 1593, 1498, 1403, 1327, 1152, 1084; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 2.39 (s, 6H), 6.79 (s, 2H), 6.94 – 6.98 (m, 2H), 7.02 – 7.05 (m, 2H), 7.22 (d, J = 8.0 Hz, 4H), 7.55 (td, J = 1.6 Hz, 3.6 Hz, 4H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 22.04, 126.51, 127.79, 128.01, 130.09, 131.31, 135.98, 144.66; **HRMS (ESI):** Calculated for $C_{20}H_{21}N_2O_4S_2$ $[M+H]^+$: 417.0942; Found: 417.0941.

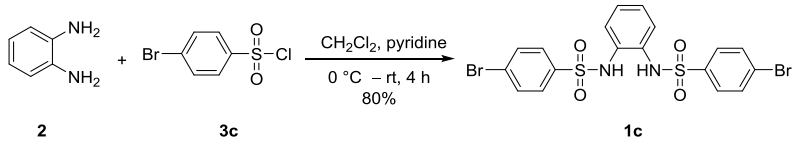
Synthesis of *N, N′*-(1, 2-phenylene)bis(4-nitrobenzenesulfonamide) (1b):



Scheme S2. Synthesis of compound **1b**.

In a 50 mL round bottom flask, *o*-phenylenediamine **2** (200 mg, 1.85 mmol) was dissolved in 10 mL of dry CH_2Cl_2 followed 0.5 mL of pyridine was added and reaction mixture was cooled to 0 ^oC. 4-nitrobenzenesulfonyl chloride **3b** (1.27 g, 3.88 mmol) dissolved in 5 mL of dry CH_2Cl_2 was added dropwisely to the reaction mixture and was stirred for 30 min at 0 ^oC. Temperature was brought to room temperature and the reaction was stirred for 4 h. Then the reaction TLC was checked to confirm for completion of the reaction. The solvent of reaction mixture was concentrated to remove CH_2Cl_2 and pyridine and then the residue was subjected to column chromatography using Ethyl acetate, Methanol and Pet ether. **Eluent-** EtOAc/MeOH/Pet ether (70:4:26). An off-white coloured solid product **1b** (760 mg, 86%) was obtained. **mp:** 255 ^oC; **IR (KBr):** ν/cm^{-1}: 3282, 3104, 1933, 1811, 1681, 1605, 1536, 1403, 1349, 1163, 1087; **^{1}H NMR (400 MHz, Acetone-d_6):** δ 7.07 – 7.12 (m, 2H), 7.15 – 7.19 (m, 2H), 7.95 (td, J = 2.4 Hz, 9.2 Hz, 4H), 8.36 (td, J = 2.4 Hz, 9.2 Hz, 4H), 8.73 (s, 2H); **^{13}C NMR (100 MHz, Acetone-d_6):** δ 125.27, 127.23, 128.70, 129.73, 131.77, 145.31, 151.47; **HRMS (ESI):** Calculated for $C_{18}H_{15}N_4O_8S_2$ $[M+H]^+$: 479.0353; Found: 479.0331.

Synthesis of *N, N′*-(1, 2-phenylene)bis(4-bromobenzenesulfonamide) (1c):

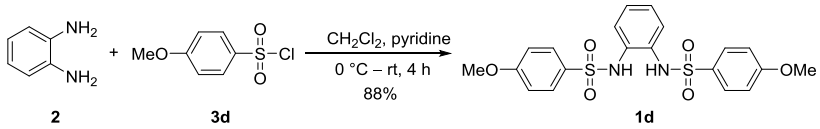


Scheme S3. Synthesis of compound **1c**.

In a 50 mL round bottom flask *o*-phenylenediamine **2** (200 mg, 1.85 mmol) was dissolved in 10 mL of dry CH_2Cl_2 and followed by addition of 0.5 mL of pyridine and reaction mixture was cooled to 0 ^oC. Then 4-bromobenzenesulfonyl chloride **3c** (0.99 g, 3.88 mmol) dissolved in 5 mL

of dry CH_2Cl_2 was added dropwisely to the reaction mixture and was stirred for 30 min at 0 ^oC .Then the temperature was brought to room temperature and the reaction was stirred for 4 h with constant stirring. Then the reaction TLC was checked to confirm for completion of the reaction. The reaction mixture was then concentrated and then the residue was subjected to column chromatography using Methanol and Chloroform. Compound **1c** was obtained as crystalline, white solid product (803.2 mg, 80%). **Eluent:** 5% MeOH/$CHCl_3$; **mp:** 224 ^oC ; **IR (KBr):** ν/cm^{-1}: 3216, 1915, 1570, 1483, 1400, 1334, 1276, 1157, 1076, 1006; **^{1}H NMR (400 MHz, DMSO-d_6):** δ 6.96 (m, 2H), 7.04 (m, 2H), 7.61 (d, J = 8.4 Hz, 4H), 7.76 (d, J = 8.4 Hz, H, 4H), 9.43 (s, 2H); **^{13}C NMR (100 MHz, DMSO-d_6):** δ 123.96, 126.35, 127.13, 128.79, 129.72, 132.36, 138.34; **HRMS (ESI):** Calculated for $C_{18}H_{15}Br_2N_2O_4S_2$ $[M+H]^+$: 544.8840, 546.8820; Found: 544.8845, 546.8822.

Synthesis of *N, N′*-(1, 2-phenylene)bis(4-methoxybenzenesulfonamide) (1d):



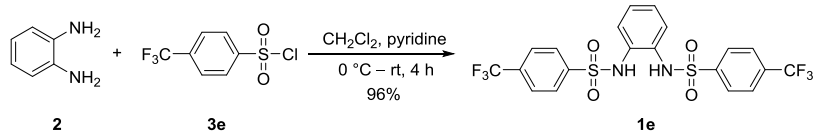
2 3d 1d

Scheme S4. Synthesis of compound **1d**.

In a 50 mL round bottom flask *o*-phenylenediamine **2** (200 mg, 1.85 mmol) of was dissolved in 10 mL of dry CH_2Cl_2, followed by 0.5 mL of pyridine was added and reaction mixture was cooled to 0 ^oC. 4-methoxybenzenesulfonyl chloride **1d** (0.76 g, 3.70 mmol) dissolved in 5 mL of dry CH_2Cl_2 was added dropwisely to the reaction mixture and was stirred for 30 min at 0 ^oC. Then the temperature was brought to room temperature and the reaction was stirred for 4 h and TLC was monitored in order to confirm the completion of reaction. The reaction mixture was then concentrated and then the residue was directly subjected to column chromatography using ethyl acetate and pet ether solvent mixture. Compound **1d** (730 mg, 88%) was obtained as crystalline white solid. **Eluent:** EtOAc /PE (40%); **mp:** 155 ^oC; **IR (KBr):** ν/cm^{-1}: 3240, 1909, 1589, 1501, 1397, 1326, 1258, 1154, 1086, 1017; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 3.84 (s, 6H), 6.81 (s, 2H), 6.86 (td, J = 3.2 Hz, 8.8 Hz, 4H), 6.95 – 6.98 (m, 2H), 7.02 – 7.06 (m, 2H), 7.59 (td, J = 3.2 Hz, 8.8 Hz, 4H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 55.77, 114.30, 126.14, 127.37, 129.86,

130.06, 131.06, 163.47; **HRMS (ESI):** Calculated for $C_{20}H_{21}N_2O_6S_2$ $[M+H]^+$:449.5226; Found: 449.5204.

Synthesis of *N, N'*-(1, 2-phenylene)bis(4-trifluoromethyl)benzenesulfonamide)(1e):



2 3e 1e

Scheme S5. Synthesis of compound **1e**.

In a 25 mL round bottom flask *o*-phenylenediamine **2** (22.09 mg, 0.20 mmol) was dissolved in 4 mL of CH_2Cl_2 followed by 0.5 mL of pyridine was added and reaction mixture was cooled to 0 ^oC. Then 4-trifluoromethylbenzenesulfonyl chloride **3e** (98 mg, 0.40 mmol) dissolved in 3 mL CH_2Cl_2 was added dropwisely to the reaction mixture and was stirred for 30 min at 0 ^oC. Then the temperature was brought to room temperature and the reaction was stirred for 4 h. Then the reaction TLC was checked to confirm for completion of the reaction. The reaction mixture was then concentrated and then the residue was subjected to column chromatography using ethyl acetate and pet ether. Compound **1e** (102 mg, 96%) was obtained as crystalline, white solid product. **Eluent:** 12% EtOAc in Pet ether **mp:** 178 ^oC; **IR (KBr):** ν/cm^{-1}: 3263, 2925, 2856, 1602, 1502, 1403, 1329, 1179, 1057, 1010; **^{1}H NMR (400 MHz, $CDCl_3$):** δ 6.98 – 6.96 (m, 2H), 7.10 – 7.13 (m, 4H), 7.71 (d, J = 8.4 Hz, 4H), 7.82 (d, J = 8 Hz, 4H); **^{13}C NMR (100 MHz, $CDCl_3$):** δ 121.83, 124.54, 126.35, 126.41, 128.25, 130.42, 134.97, 141.86; **HRMS (ESI):** Calculated for $C_{20}H_{15}F_6N_2O_4S_2$ $[M+H]^+$: 525.0377; Found: 525.0368.

IV. Ion Transport Activity Study

HEPES Buffer, HPTS and Stock Solution Preparation: Solid HEPES and NaCl were dissolved in autoclaved water to prepare HEPES buffer (10 mM) with NaCl (100 mM), followed by dropwise addition of NaOH solution to adjust pH = 7.0. Solid HPTS dye was dissolved in above 1 mL of aforementioned buffer solution to give 1 mM solution of 10 mM HEPES containing NaCl (100 mM) of pH = 7.0. Stock solutions of all bis(sulfonamide) derivatives were prepared by dissolving in DMSO of HPLC grade.

Preparation of EYPC-LUVs⊃HPTS: EYPC-LUVs⊃HPTS (~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0 and outside: 10 mM HEPES, 100 mM NaCl, pH = 7.0) were prepared following reported protocol. [S2, S3]

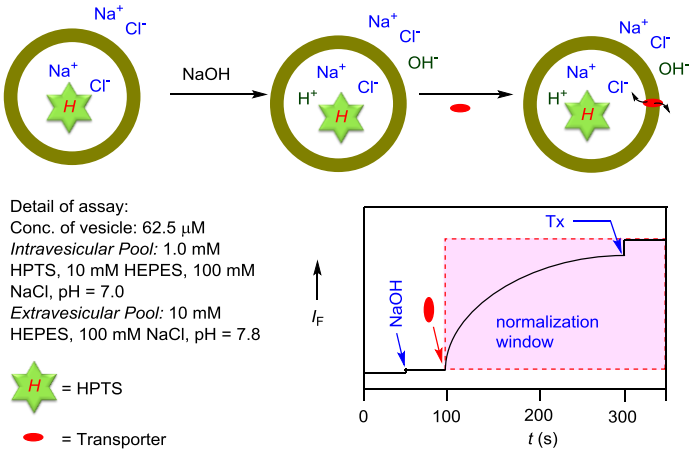


Fig. S1 Representation of ion transport activity assay using EYPC vesicles, assay detail and representation of ion transport experiment using fluorescence.

Ion Transport Activity Assay Protocol: 1975 μL of HEPES buffer containing 10 mM HEPES, 100 mM NaCl (pH = 7.0) was taken in a clean fluorescence cuvette followed by addition of 25 μL of EYPC-LUVs⊃HPTS in the same cuvette and was placed on a magnetic stirrer equipped with the fluorescence instrument (at t = 0 s). Fluorescence intensity of pH sensitive dye HPTS, F_t was monitored at λ_{em} = 510 nm (λ_{ex} = 450 nm). After that 20 μL of 0.5 M NaOH was added to create pH gradient between the intra and extra vesicular system into the same cuvette at t = 20 s (Fig. S1). bis(sulfonamide) derivatives were added at t = 100 s and finally at t = 300 s, 25 μL of 10% Triton X-100 was added to lyze all vesicles which resulted in destruction of pH gradient (Fig. S1) and saturation in fluorescence emission intensity was observed.

The time axis was normalized according to Equation S1:

$$t = t - 100 \qquad \text{(S1)}$$

Now the time of bis(sulfonamide) compound addition can be normalized to t = 0 s and time of Triton-X 100 addition was normalized to t = 200 s.

Fluorescence time courses (F_t) were normalized to fractional emission intensity I_F using Equation S2.

$$\% \, Fl \, Intensity \, (I_F) = [(F_t - F_0) / (F_\infty - F_0)] \times 100 \qquad \text{(S2)}$$

Where F_0 = Fluorescence intensity just before the bis(sulfonamide) compound addition (at t = 0 s). F_∞ = Fluorescence intensity at saturation after complete leakage (at t = 330 s). F_t = Fluorescence intensity at time t. Concentration dependent experiments were carried out by increasing concentration of bis(sulfonamide) compounds (**1a** – **1e**). Change of HPTS fluorescence emission intensity was monitored with time and addition of bis(sulfonamide) compounds resulted in destruction of pH gradient via either Na^+/OH^- symport or Na^+/H^+ antiport mechanism (Fig. S2).

The concentration profile data were further analyzed by Hill Equation to get the Effective concentration (EC_{50}) and Hill Coefficient (n), (Equation S3).

$$Y = Y_\infty + (Y_0 - Y_\infty) / [1 + (c / EC_{50})^n] \qquad \text{(S3)}$$

Where, Y_0 = Fluorescence intensity just before the bis(sulfonamide) compounds (**1a** – **1e**) addition (at t = 0 s). Y_∞ = Fluorescence intensity with excess bis(sulfonamide) compounds (**1a** – **1e**) concentration, c = Concentration of bis(sulfonamide) compounds (**1a** – **1e**) (Fig. S3).

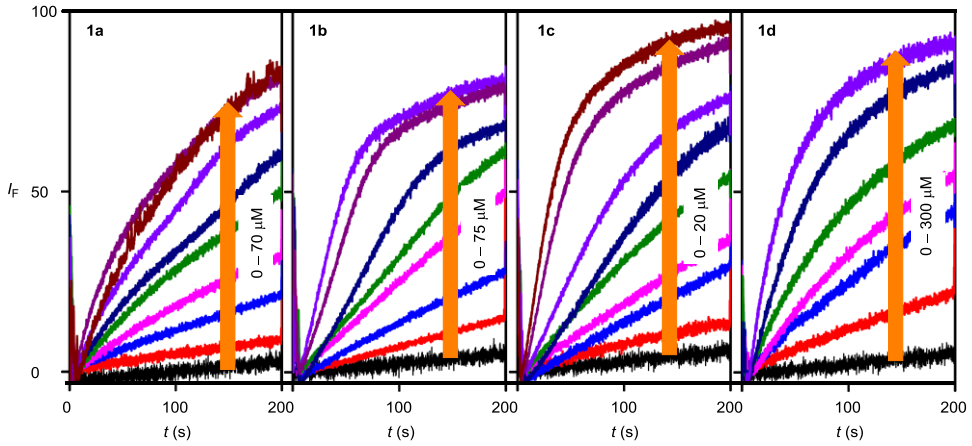


Fig. S2 Ion transport assay of bis(sulfonamide) derivatives **1a ─ 1d** using EYPC LUVs⊃HPTS.

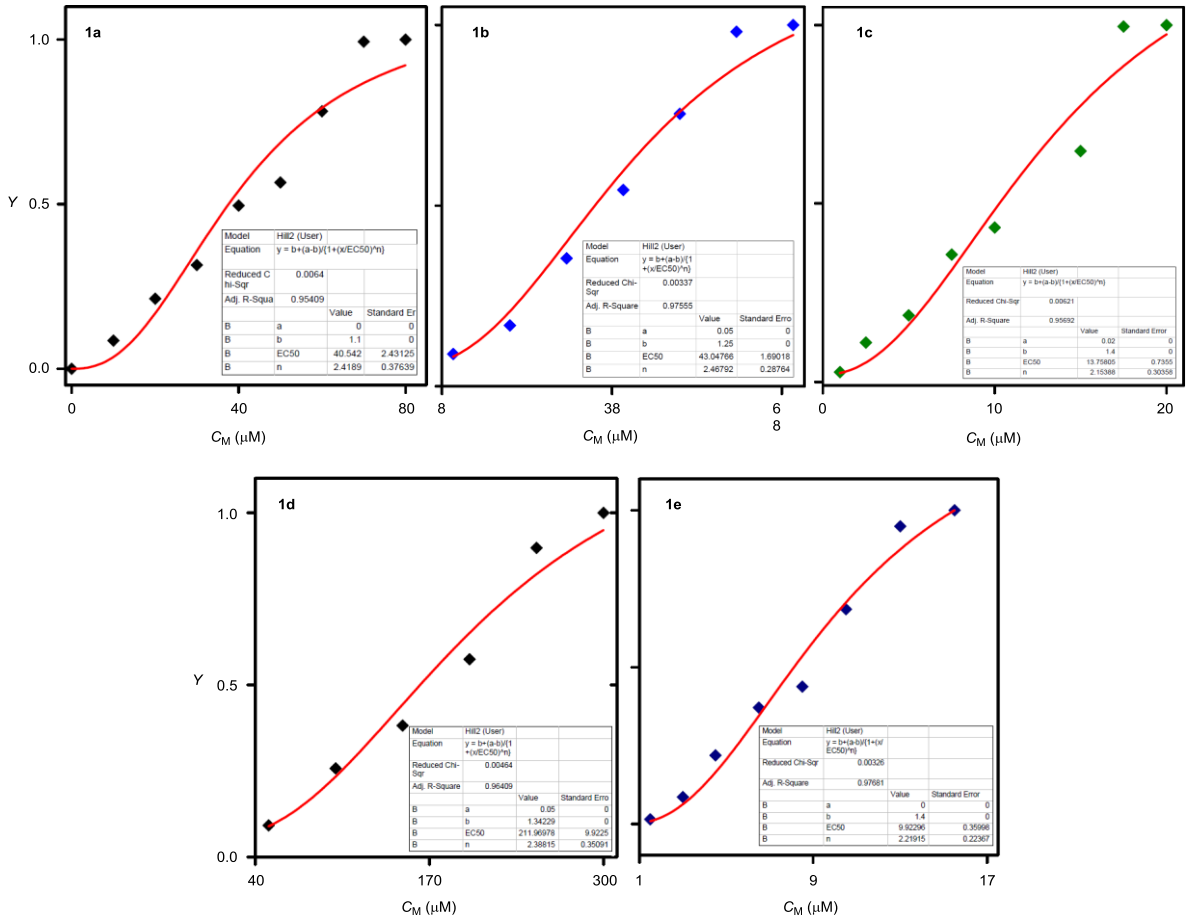


Fig. S3 Representation of Hill plots for bis(sulfonamide) compounds (**1a** – **1e**).

Determination of Ion Selectivity by HPTS assay:

Buffer and Stock Solution Preparation: HEPES buffers with all salts were prepared by dissolving solid HEPES (10 mM) followed by addition of appropriate salt (100 mM) in autoclaved water and adjustment of pH (pH = 7.0) was done by dropwise addition of NaOH solution.

Preparation of EYPC-LUVs⊃HPTS for Cation Selectivity: EYPC-LUVs⊃HPTS (~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0 and outside: 10 mM HEPES, 100 mM NaCl, pH = 7.0) were prepared following reported protocol. [S3- S5]

Cation Selectivity Assay: In a clean fluorescence cuvette 1975 μL of different HEPES buffer solutions (10 mM HEPES, 100 mM MCl, pH = 7.0; where, M^+ = Li^+, Na^+, K+, Rb^+ and Cs^+)

were taken followed by addition of 25 μL of EYPC-LUVs⊃HPTS vesicle in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at t = 0 s). The time course of HPTS fluorescence intensity, F_t was monitored at λ_{em} = 510 nm (λ_{ex} = 450 nm). At t = 20 s, 20 μL of 0.5 M NaOH was added to the cuvette to make the pH gradient between the intra and extra vesicular system. The bis(sulfonamide) compounds (**1c** and **1e**) were added at t = 100 s and at t = 300 s, 10% Triton X-100 (25 μL) was added to lyze all vesicles for complete destruction of pH gradient.

For data analysis and comparison, time (X-axis) was normalized between the point of transporter addition (*i.e.* t = 100 s was normalized to t = 0 s) and end point of experiment (*i.e.* t = 300 s was normalized to t = 200 s). Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation S2.

Preparation of EYPC-LUVs⊃HPTS for Anion Selectivity: EYPC-LUVs⊃HPTS (~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0 and outside: 10 mM HEPES, 100 mM NaCl, pH = 7.0) were prepared following reported protocol. [S3-S5]

Anion Selectivity Assay: In a clean fluorescence cuvette 1975 μL of HEPES buffer (10 mM HEPES, 100 mM NaX, at pH = 7.0; where, X^- = F^-, Cl^-, Br^- and I^-) was added followed by addition of 25 μL of EYPC-LUVs⊃HPTS vesicle in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at t = 0 s). HPTS fluorescence emission intensity (F_t) was monitored with time at λ_{em} = 510 nm (λ_{ex} = 450 nm). 20 μL of 0.5 M NaOH was added to the cuvette at t = 20 s to make the pH gradient between the intra and extra vesicular system. The bis(sulfonamide) compounds (**1c** and **1e**) were added at t = 100 s and at t = 300 s, 25 μL of 10% Triton X-100 was added to lyze all vesicles for complete destruction of pH gradient.

For data analysis and comparison, time (X-axis) was normalized between the point of transporter addition (*i.e.* t = 100 s was normalized to t = 0 s) and end point of experiment (*i.e.* t = 300 s was normalized to t = 200 s) using Equation S1. Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation S2.

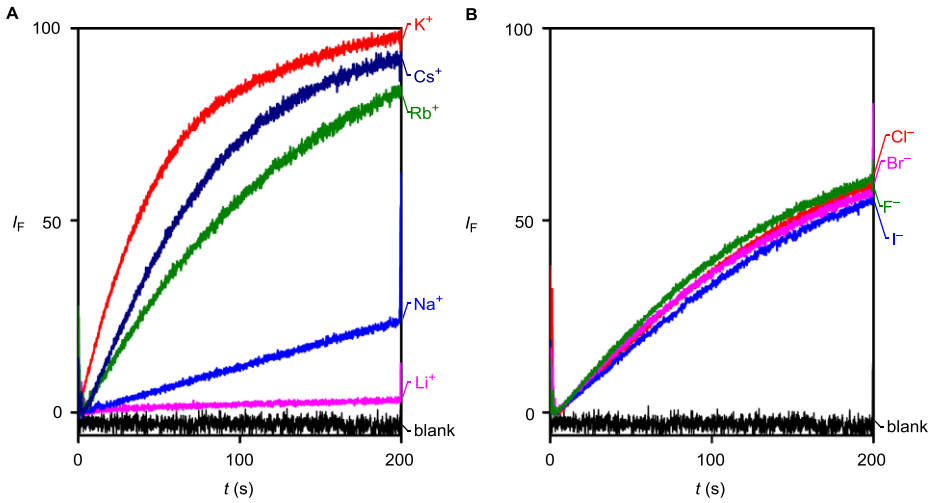


Fig. S4 (**A**) Cation selectivity of bis(sulfonamide) derivatives **1c** (5 μM) and (**B**) anion selectivity of **1c** (14 μM) using EYPC LUVs⊃HPTS.

Preparation of EYPC-LUVs⊃HPTS for Membrane Polarization Assay:[S6] EYPC-LUVs⊃HPTS (~ 5.0 mM EYPC, inside: 1 mM HPTS, 10 mM HEPES, 100 mM NaCl, pH = 7.0 and outside: 10 mM HEPES, 100 mM NaCl, pH = 7.0) were prepared following reported protocol.

Membrane Polarization Assay: 1975 μL of HEPES buffer containing 10 mM HEPES, 100 mM NaCl (or 100 mM KCl in case of K^+ transport) at pH = 7.0 was taken in a clean fluorescence cuvette followed by addition of 25 μL of EYPC-LUVs⊃HPTS in the same cuvette and was placed on a magnetic stirrer equipped with the fluorescence instrument (at t = 0 s). Fluorescence intensity of pH sensitive dye HPTS, F_t was monitored at λ_{em} = 510 nm (λ_{ex} = 450 nm). bis(sulfonamide) compound **1e** was added at t = 100 s and at t = 350 s, 20 μL 0.5 M NaOH was added to create pH gradient between intravesicular and extravesicular solution. Finally at t = 600 s, 25 μL of 10% Triton X-100 was added to lyze all vesicles which resulted in destruction of pH gradient and saturation in fluorescence emission intensity was observed.

Preparation of EYPC-LUVs at pH = 7.0 for Membrane Polarisation Experiment With Safranin O Dye: [S6, S7] 1 mL egg yolk phosphatidylcholine (EYPC) (25 mg/mL in $CHCl_3$) lipid was taken in a 5 mL clean and dry small round bottomed flask. A thin transparent film of lipid

was prepared by purging nitrogen with continuous rotation. The resulting transparent film was kept in high vacuum for at least 5 h to remove trace amount of $CHCl_3$ for drying. After that transparent film was hydrated with 1 mL of aforementioned buffer (10 mM HEPES buffer, 100 mM KCl, pH = 7.0) for 1 h with occasional vortexing of 4-5 times and then subjected to freeze-thaw cycle (≥ 15 times). Extrusions were done 19 times (must be an odd number) by a Mini-extruder equipped with a polycarbonate membrane (pore diameter of 200 nm) and obtained vesicle was diluted to 6 mL with phosphate buffer (10 mM HEPES, 100 mM KCl, pH = 7.0) to give EYPC-LUVs, inside: 10 mM HEPES, 100 mM KCl, pH = 7.0 and outside: 10 mM HEPES, 100 mM KCl, pH = 7.0. There is no HPTS dye inside vesicle and for that size exclusion chromatography was not performed.

Membrane Polarisation Assay With Safranin O Dye: 1900 μL of HEPES buffer (10 mM, 100 mM NaCl, pH = 7.0) was taken into a fluorescence quartz cuvette and 100 μL of stock vesicle solution was suspended into it and placed in slowly stirring condition by a magnetic stirrer equipped with the fluorescence instrument (at t = 0 s). Safranin O (60 nM) was then added in the cuvette and Fluorescence emission intensity was monitored at an excitation of 522 nm and emission of 581 nm with time. After 100 sec, compound **1e** (5 μM) was added and the fluorescence monitored for 400 s

Preparation of EYPC-LUVs⊃HPTS at Different pH: Vesicles were prepared following reported protocol in different HEPES buffer solutions of different pH (both intravesicular and extravesicular pH = 8.0, 7.0, 5.5).

Selectivity Assay: All selectivity assays were carried out in the aforementioned procedure without addition of NaOH.

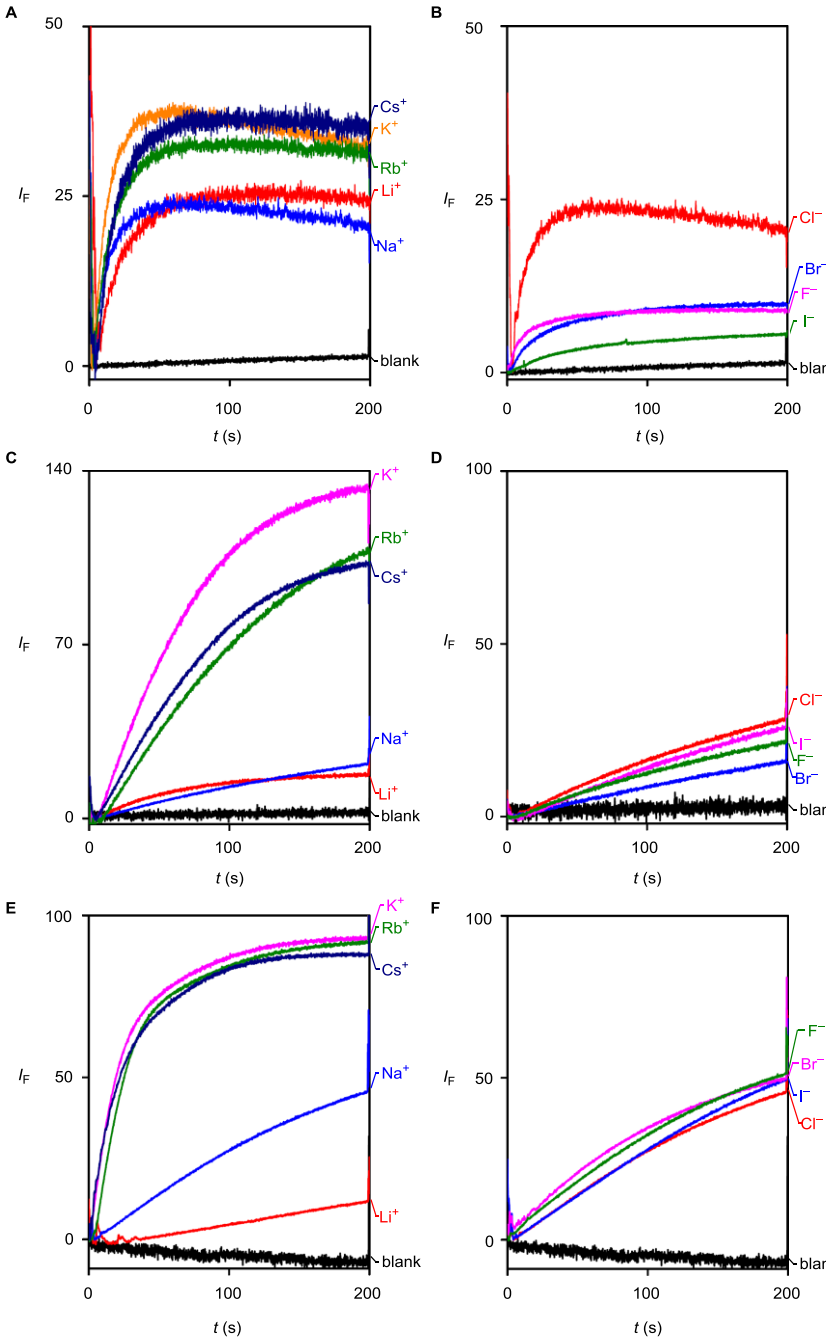


Fig. S5 Cation selectivity of bis(sulfonamide) **1e** (**A, C, E**) at pH = 5.5, 7.0, 8.0 respectively and anion selectivity of **1e** (**B, D, F**) at pH = 5.5, 7.0, 8.0 at *c* = 7.5 μM respectively using EYPC LUVs⊃HPTS without adding NaOH.

Buffer Solution Preparation for Chloride Ion Selective Electrode Assays: 5 mM sodium phosphate buffer was prepared with three different pH (pH = 5.8, 7.0, 8.0) with $NaNO_3$ salt. pH

of the buffer was adjusted by adding monobasic or dibasic sodium phosphate solution (5 mM) as per requirement. Similarly, 5 mM sodium phosphate buffer was prepared with three different pH (pH = 5.8, 7.0, 8.0) with NaCl salt.

Preparation of EYPC-LUVs at pH = 7.0: 1 mL egg yolk phosphatidylcholine (EYPC) (25 mg/mL in $CHCl_3$) lipid was taken in a 5 mL clean and dry small round bottomed flask. A thin transparent film of lipid was prepared by purging nitrogen with continuous rotation. The resulting in transparent film which was dried in high vacuum for at least 5 h to remove trace amount of $CHCl_3$ for drying. After that transparent film was hydrated with 1 mL of aforementioned buffer (5 mM phosphate buffer, 500 mM NaCl, pH = 7.0) for 1 h with occasional vortexing of 4-5 times and then subjected to freeze-thaw cycle (≥ 15 times). Extrusions were done 19 times (must be an odd number) by a Mini-extruder equipped with a polycarbonate membrane (pore diameter of 200 nm). All extravesicular ions were removed by dialysis against phosphate buffer (500 mM $NaNO_3$) for atleast 4 – 5 h and obtained vesicle was diluted to 2 mL with phosphate buffer (5 mM sodium phosphate, 500 mM $NaNO_3$, pH = 7.0) to give EYPC-LUVs, inside: 5 mM sodium phosphate, 500 mM NaCl, pH = 7.0 and outside: 5 mM sodium phosphate, 500 mM $NaNO_3$, pH = 7.0.

Vesicles of pH = 5.8 and 8.0 were prepared following abovementioned protocol in different phosphate buffer solutions of different pH (both intravesicular and extravesicular pH = 8.0, 5.8).

Activity Assay: In a clean glass vial 1950 μL of phosphate buffer (5 mM sodium phosphate, 500 mM $NaNO_3$) was added followed by addition of 50 μL of EYPC-LUVs vesicle in slowly stirring condition by a magnetic stirrer. 20 μM of **1e** was added to the glass vial at t = 30 s and chloride efflux was monitored by chloride ion selective electrode after each 10 s and finally at t = 300 s, 25 μL of 10% Triton X-100 was added to lyze all vesicles. Fluorescence intensities (F_t) were normalized to fractional emission intensity I_F using Equation S2. The EC_{50} was calculated from concentration dependent plot at pH = 5.8 and followed by fitting into Hill equation (Equation S3). Chloride efflux activity at 15 μM concentration was fitted in asymptotic equation in the Origin 8.5 to obtain initial rate (I_R).

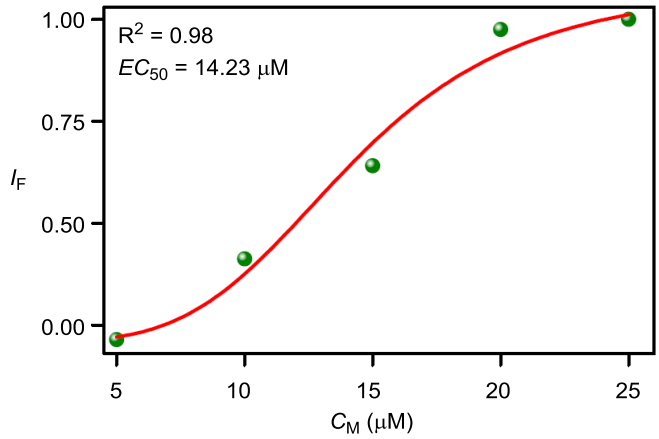


Fig. S6 Representation of Hill plot for bis(sulfonamide) **1e** at pH = 5.8 using EYPC-LUVs.

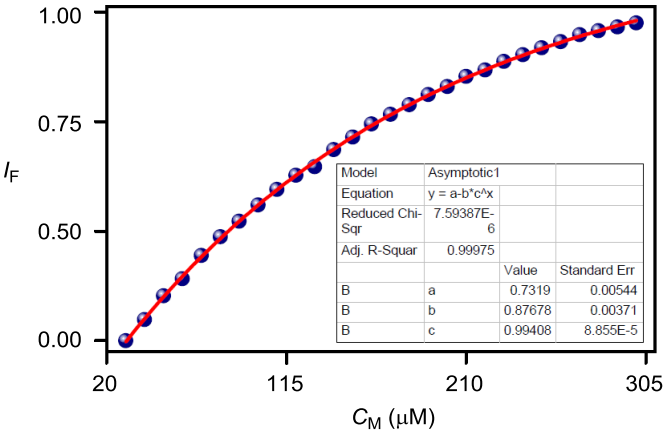


Fig. S7 Asymptotic fitting to obtain initial rate of bis(sulfonamide) **1e** at 15 μM concentration using EYPC-LUVs.

V. Theoretical Calculations: [S8- S9]

Table S1. Atomic coordinates calculated for lowest energy conformation of [(**1e**)$_2$•K$^+$] (Fig. 7) obtained from DFT B3LYP/6-311G (d,p) geometry optimization.

Symbolic Z-matrix:

Charge = -3 Multiplicity = 1

Atom	Atom Type	x	y	z
1	C	-1.8208	4.7865	2.844
2	C	-1.0865	4.4489	3.9779
3	C	-0.0106	3.5709	3.8675
4	C	0.3783	3.0313	2.6303
5	C	-0.3766	3.3787	1.4637
6	C	-1.4739	4.2461	1.6088
7	N	0	2.7885	0.2434
8	S	-0.1248	3.6014	-1.1108
9	N	1.4276	2.1034	2.4662
10	S	2.8308	2.3033	3.1575
11	O	2.8492	2.6196	4.6115
12	C	3.5699	3.8226	2.4188
13	O	3.7265	1.2071	2.7391
14	O	-1.4861	3.7684	-1.6894
15	C	0.7286	2.4535	-2.2383
16	O	0.6892	4.852	-1.1607
17	C	3.0815	4.3421	1.2243
18	C	3.6625	5.4817	0.6697
19	C	4.7475	6.0862	1.3049
20	C	5.2352	5.565	2.5106
21	C	4.6406	4.442	3.0684
22	C	0.2628	2.3607	-3.5489
23	C	0.9671	1.6105	-4.4813

24	C	2.1353	0.9448	-4.1012
25	C	2.5848	1.0163	-2.7817
26	C	1.883	1.7764	-1.8495
27	C	2.8794	0.158	-5.1316
28	F	2.1261	-0.8294	-5.6913
29	F	3.9956	-0.4333	-4.6565
30	F	3.2789	0.9355	-6.1837
31	C	5.3567	7.3323	0.7542
32	F	5.1378	7.5001	-0.5701
33	F	6.7112	7.3759	0.9269
34	F	4.889	8.4661	1.3561
35	H	-2.673	5.4575	2.9157
36	H	-1.3548	4.8515	4.9512
37	H	0.5667	3.2928	4.7401
38	H	-2.0554	4.4865	0.7265
39	H	2.2352	3.8799	0.7322
40	H	3.2525	5.8814	-0.2495
41	H	6.0767	6.0405	3.0027
42	H	4.983	4.0405	4.0144
43	H	-0.6577	2.8665	-3.8107
44	H	0.5978	1.5272	-5.4966
45	H	3.4726	0.4761	-2.4796
46	H	2.214	1.835	-0.8199
47	C	1.0865	-4.4489	3.9779
48	C	1.8208	-4.7865	2.844
49	C	1.4739	-4.2461	1.6088
50	C	0.3766	-3.3787	1.4637
51	C	-0.3783	-3.0313	2.6303
52	C	0.0106	-3.5709	3.8675
53	N	-1.4276	-2.1034	2.4662

54	S	-2.8308	-2.3033	3.1575
55	N	0	-2.7885	0.2434
56	S	0.1248	-3.6014	-1.1108
57	O	1.4861	-3.7684	-1.6894
58	C	-0.7286	-2.4535	-2.2383
59	O	-0.6892	-4.852	-1.1607
60	O	-2.8492	-2.6196	4.6115
61	C	-3.5699	-3.8226	2.4188
62	O	-3.7265	-1.2071	2.7391
63	C	-0.2628	-2.3607	-3.5489
64	C	-0.9671	-1.6105	-4.4813
65	C	-2.1353	-0.9448	-4.1012
66	C	-2.5848	-1.0163	-2.7817
67	C	-1.883	-1.7764	-1.8495
68	C	-3.0815	-4.3421	1.2243
69	C	-3.6625	-5.4817	0.6697
70	C	-4.7475	-6.0862	1.3049
71	C	-5.2352	-5.565	2.5106
72	C	-4.6406	-4.442	3.0684
73	C	-5.3567	-7.3323	0.7542
74	F	-5.1378	-7.5001	-0.5701
75	F	-6.7112	-7.3759	0.9269
76	F	-4.889	-8.4661	1.3561
77	C	-2.8794	-0.158	-5.1316
78	F	-3.2789	-0.9355	-6.1837
79	F	-2.1261	0.8294	-5.6913
80	F	-3.9956	0.4333	-4.6565
81	H	1.3548	-4.8515	4.9512
82	H	2.673	-5.4575	2.9157
83	H	2.0554	-4.4865	0.7265

84	H	-0.5667	-3.2928	4.7401
85	H	0.6577	-2.8665	-3.8107
86	H	-0.5978	-1.5272	-5.4966
87	H	-3.4726	-0.4761	-2.4796
88	H	-2.214	-1.835	-0.8199
89	H	-2.2352	-3.8799	0.7322
90	H	-3.2525	-5.8814	-0.2495
91	H	-6.0767	-6.0405	3.0027
92	H	-4.983	-4.0405	4.0144
93	K	0	0	1.2797

VI. Crystal Structure Parameters:[S10]

The bis(sulfonamide) compounds **1c** (CCDC No. 1525986) and **1e** (CCDC No. 1525987) were crystallized from chloroform/MeOH (1:1) mixture at room temperature. Single-crystal X-ray data of both compounds were collected at 200 K on a Bruker KAPPA APEX II CCD Duo diffractometer (operated at 1500 W power: 50 kV, 30 mA) using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The data integration and reduction were processed with SAINT software. A multi-scan absorption correction was applied to the collected reflections. The structures were solved by the direct method using SHELXTL and were refined on F^2 by full-matrix least-squares technique using the SHELXL-97 program package within the WINGX programme. All non-hydrogen atoms were refined anisotropically and all hydrogen atoms were located in successive difference Fourier maps and were treated as riding atoms using SHELXL default parameters. The structures were examined by using the *Adsym* subroutine of PLATON to assure that no additional symmetry could be applied to the models. CCDC numbers for bis(sulfonamide) compounds **1c** and **1e** are 1525986, 1525987 respectively which contain the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.

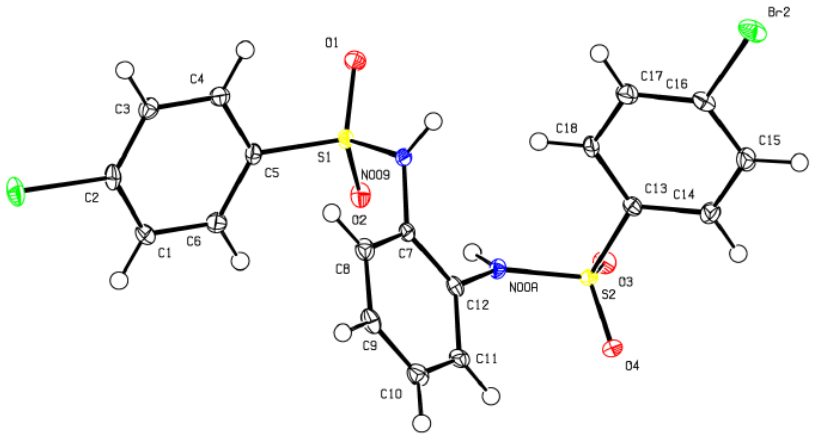


Fig. S8 ORTEP diagram of compound **1c**.

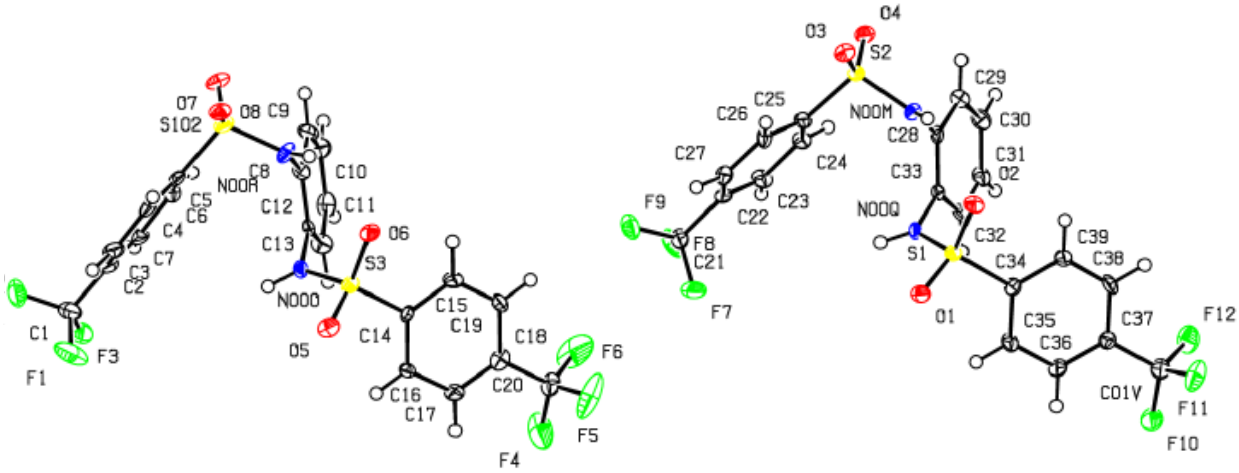


Fig. S9 ORTEP diagram of compound **1e**.

VII. NMR Spectra:

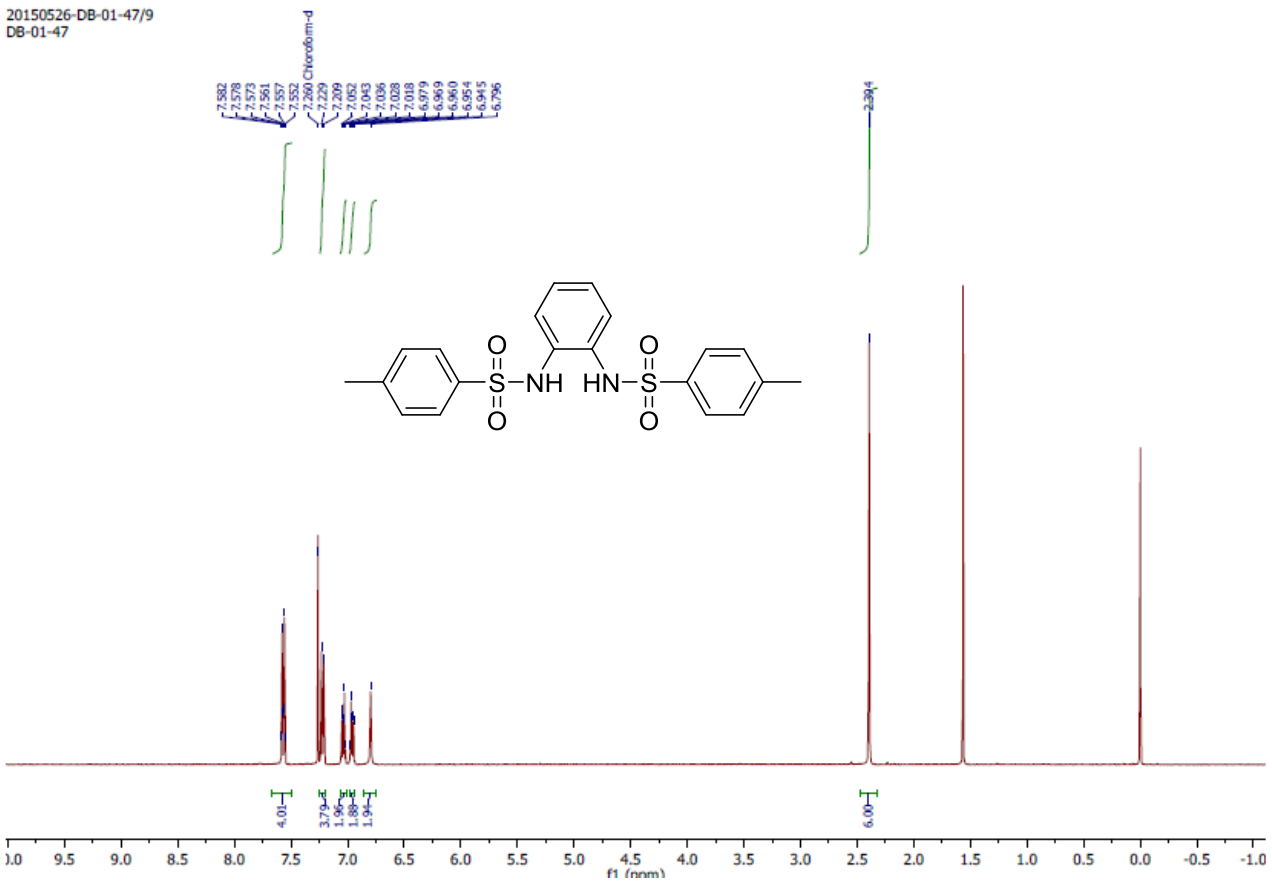


Fig. S10 ^{1}H NMR spectrum of compound **1a** in $CDCl_3$.

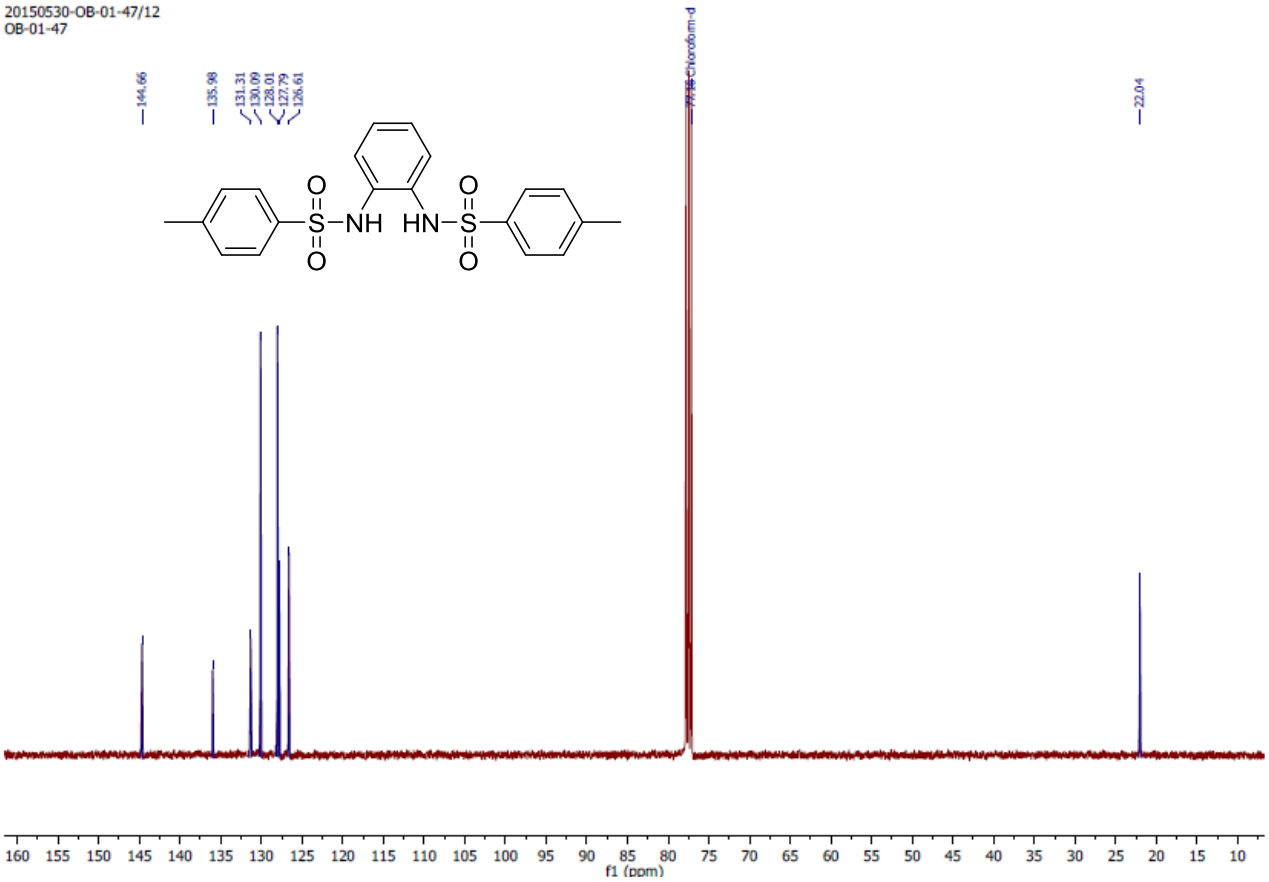


Fig. S11 ^{13}C NMR spectrum of compound **1a** $CDCl_3$.

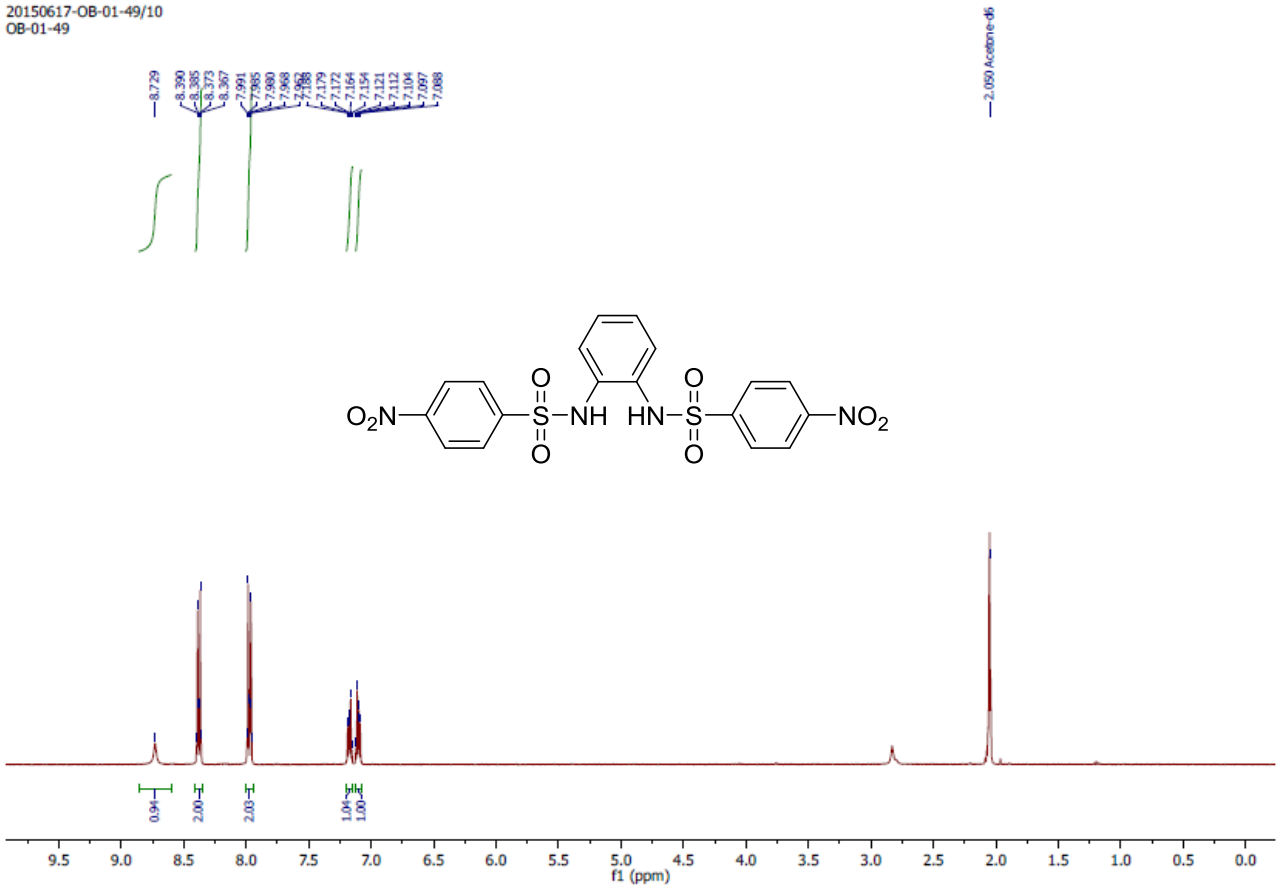


Fig. S12 ^{1}H NMR spectrum of compound **1b** in Acetone-d_6.

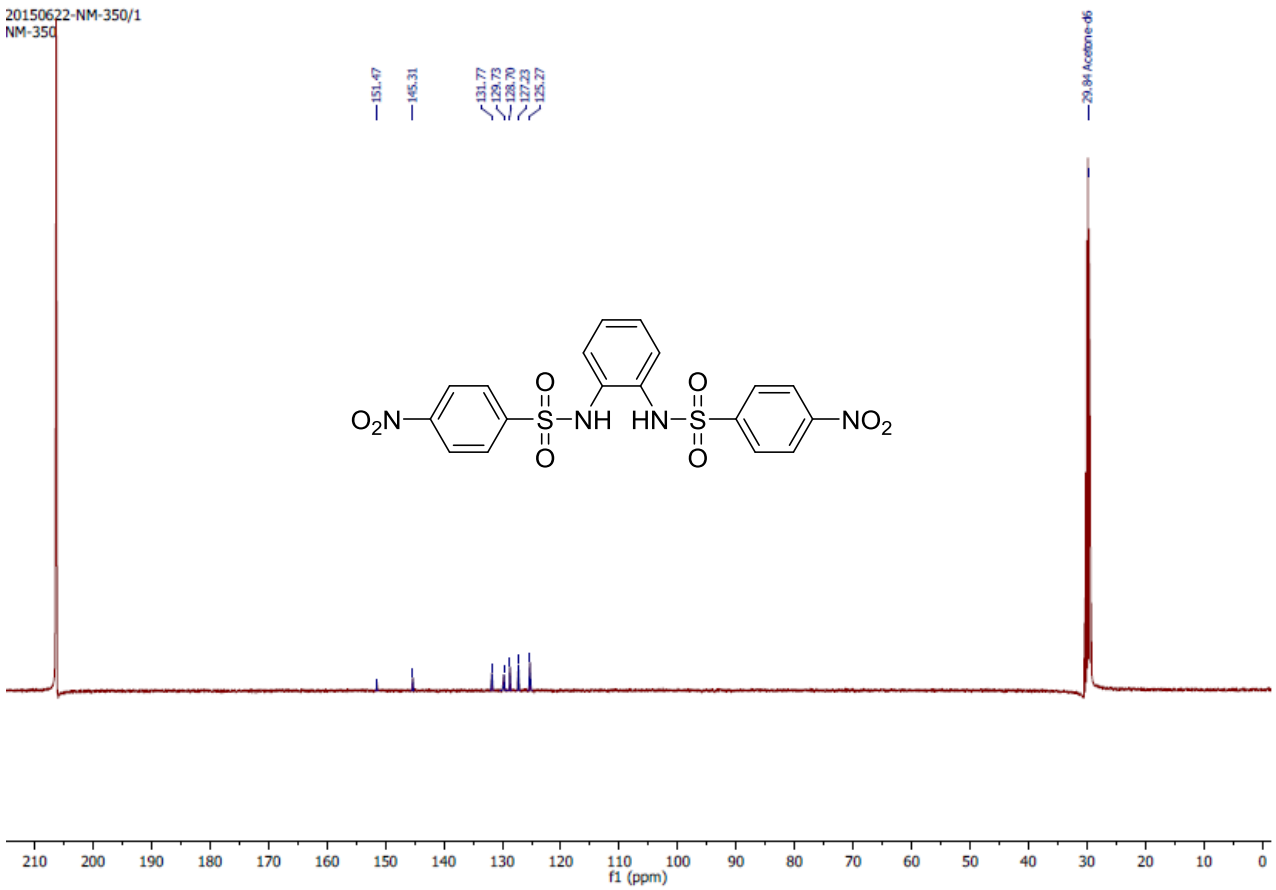


Fig. S13 ^{13}C NMR spectrum of compound **1b** in Acetone-d_6.

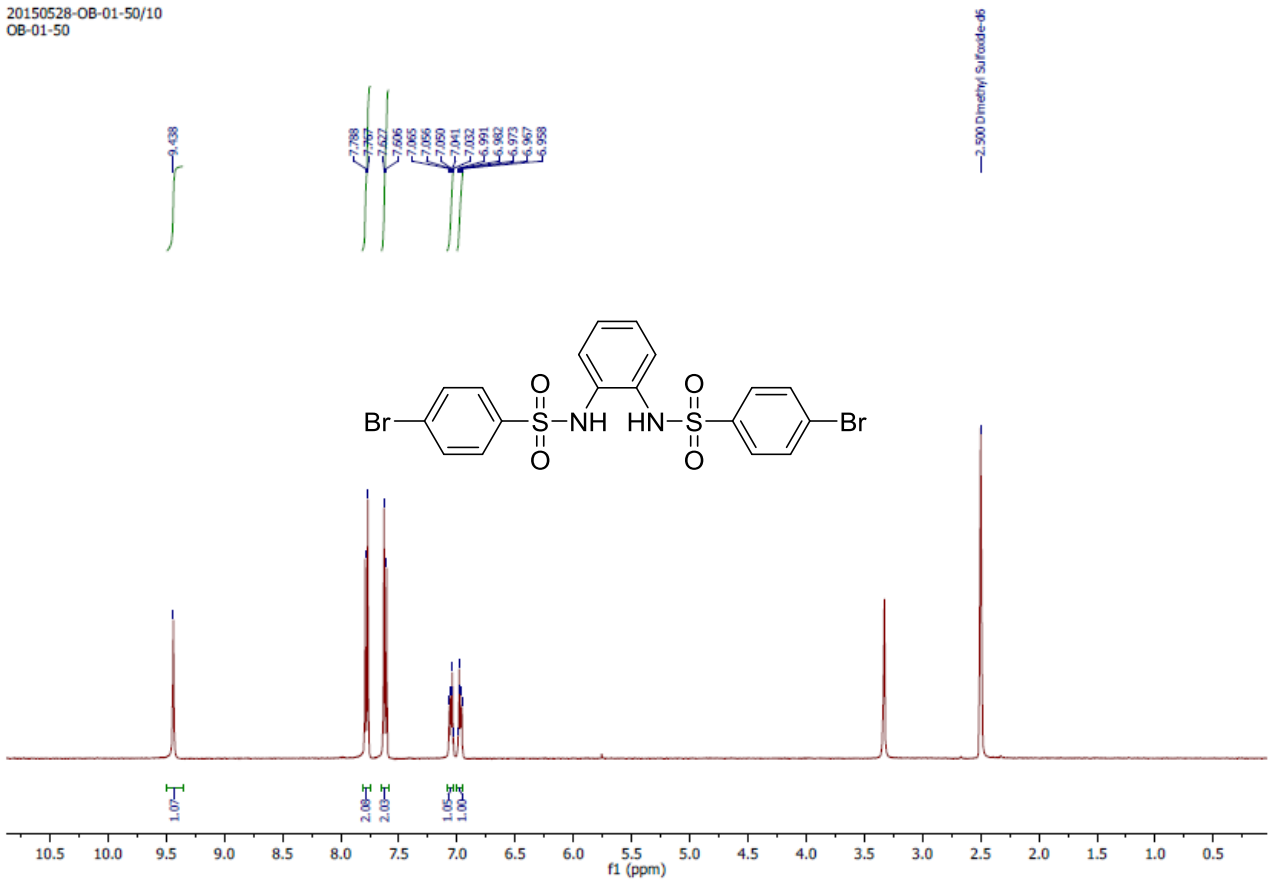


Fig. S14 ^{1}H NMR spectrum of compound **1c** in DMSO-d_6.

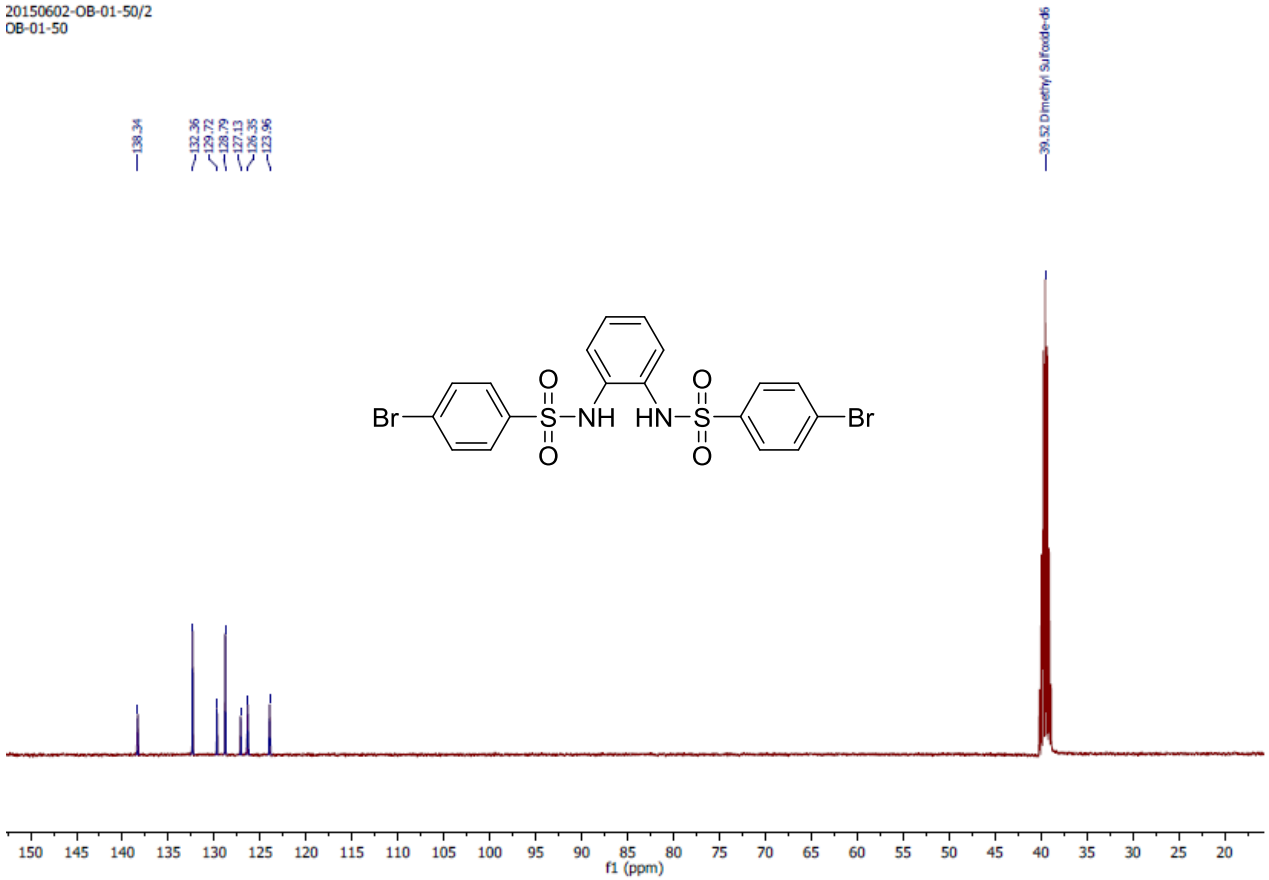


Fig. S15 ^{13}C NMR spectrum of compound **1c** in DMSO-d_6.

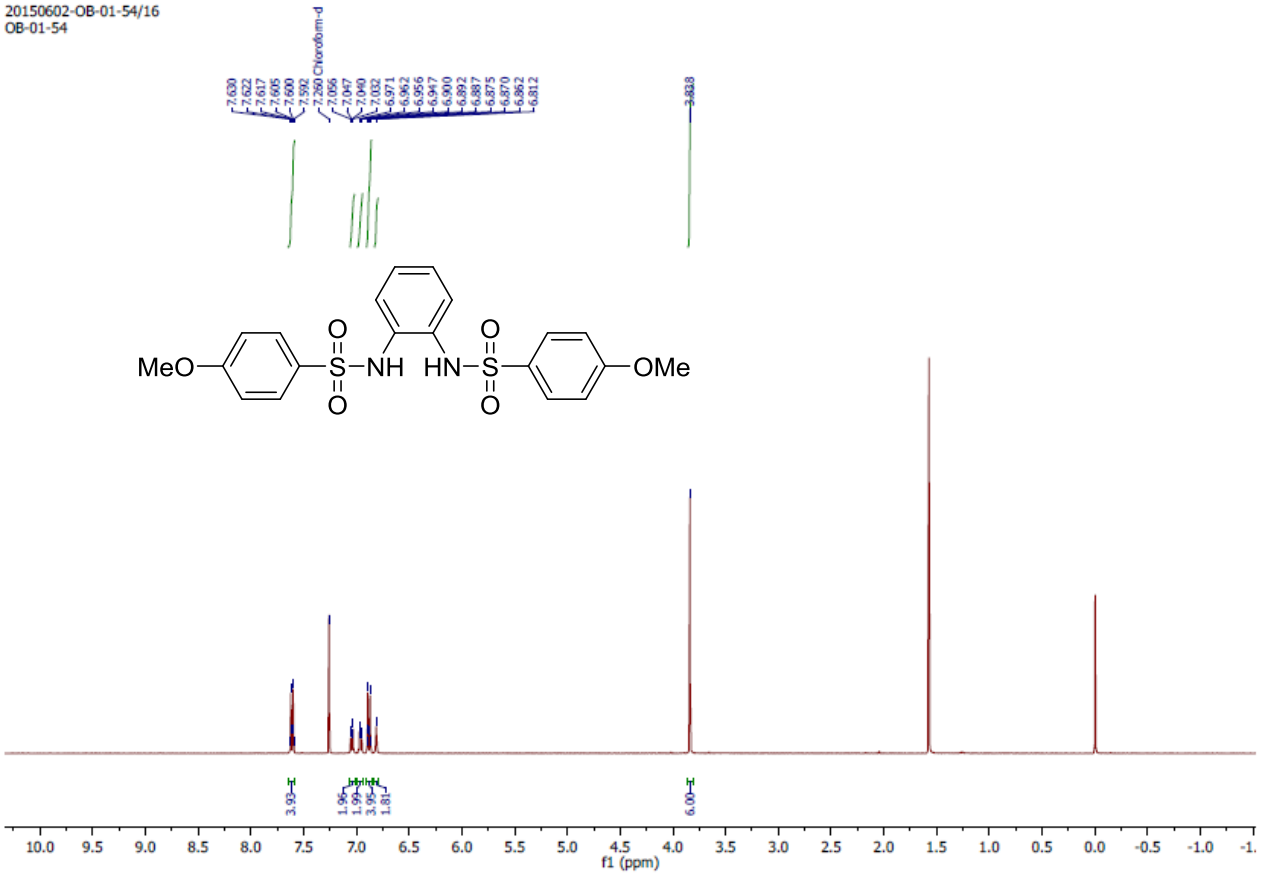


Fig. S16 ^{1}H NMR spectrum of compound **1d** in $CDCl_3$.

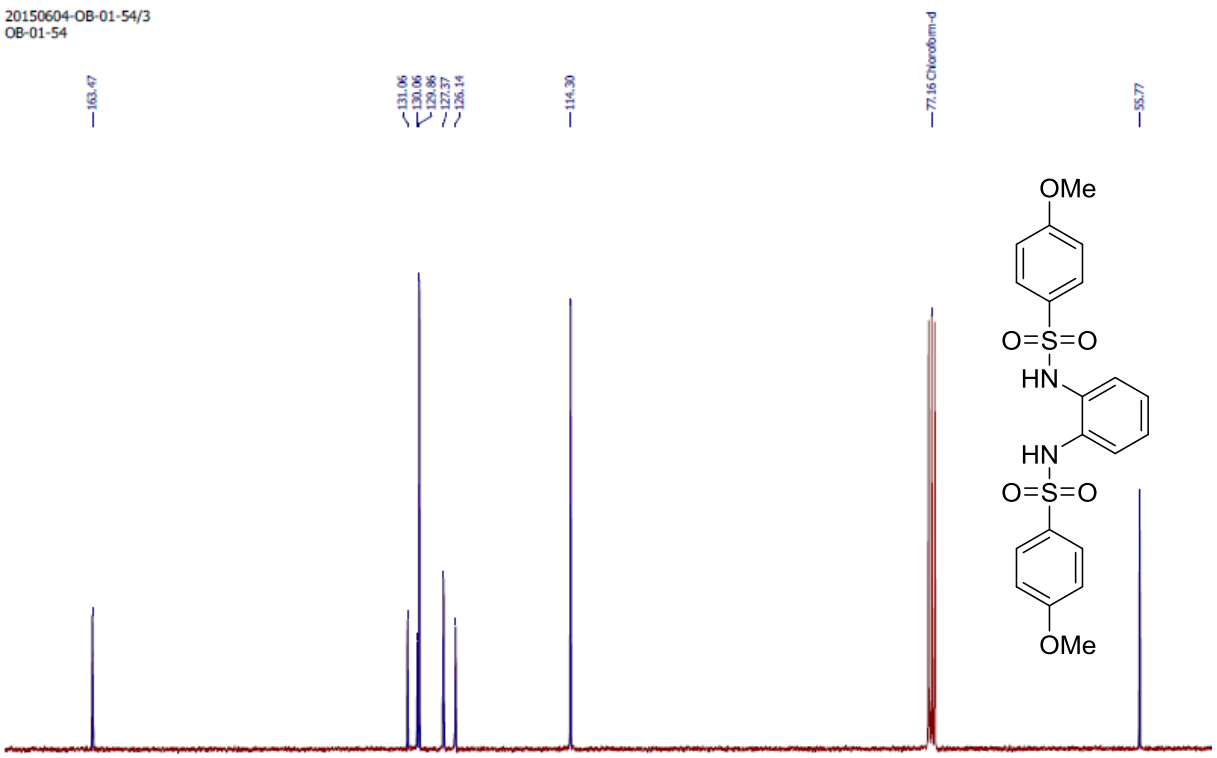


Fig. S17 ^{13}C NMR spectrum of compound **1d** $CDCl_3$.

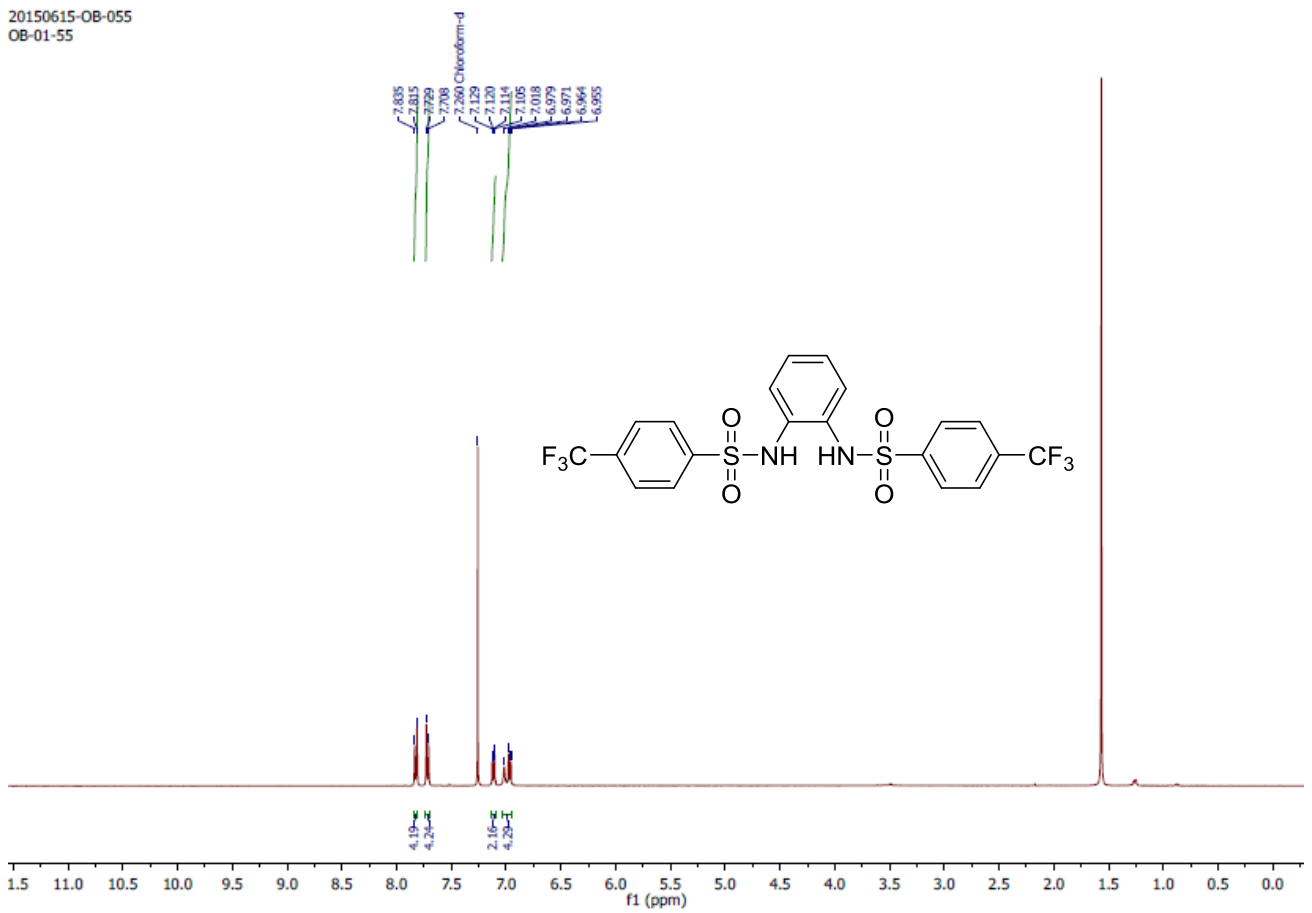


Fig. S18 ^{1}H NMR spectrum of compound **1e** $CDCl_3$.

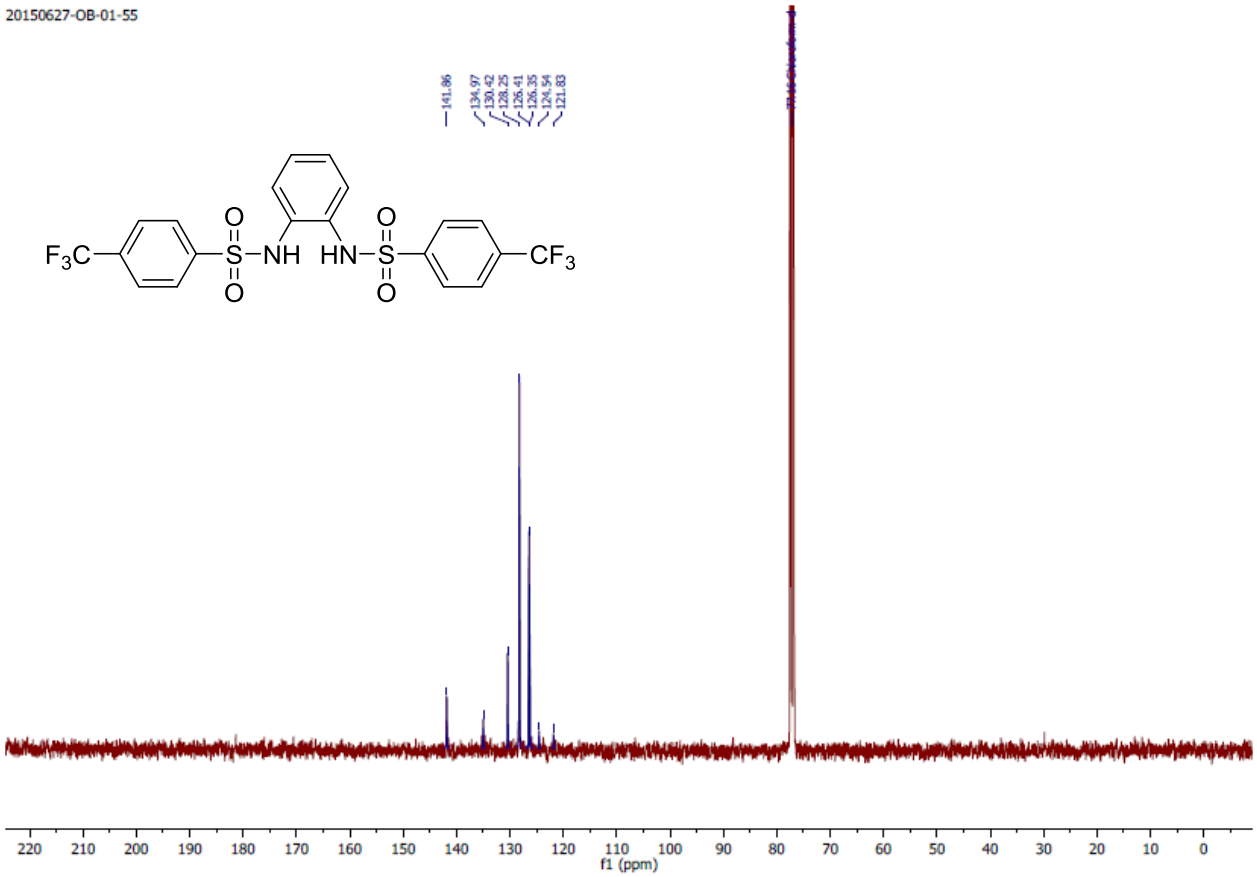


Fig. S19 ^{13}C NMR spectrum of compound **1e** $CDCl_3$.

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