

Supporting Information for

Photo-modulation of Proton / Water Transmembrane Transport through bis(imidazole-amide)-tetrafluoro-azobenzene switch

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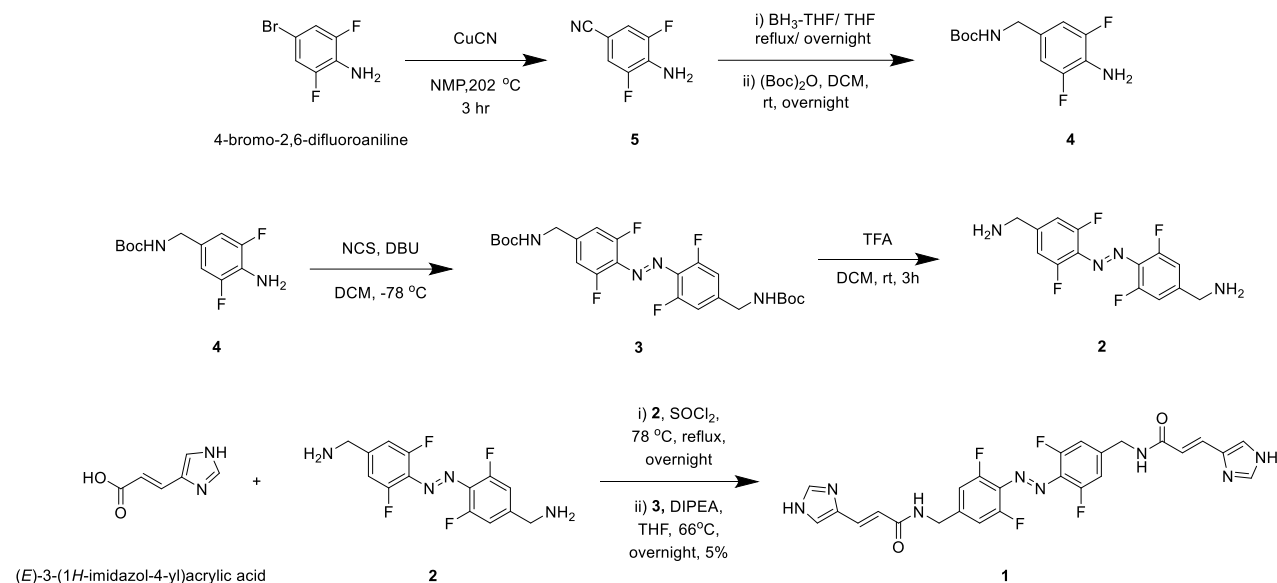
1. Materials and Methods

All reagents and solvents were obtained from commercial sources including Sigma Aldrich and VWR and were used without further purification. 4-bromo-2,6-difluoroaniline, E-3-(1H-imidazol-4-yl)acrylic acid, Sodium Chloride, Sodium Methoxide, Sodium Iodide, Sodium Nitrate, Sodium Bromide, Potassium Chloride, Lithium Chloride, Rubidium Chloride, Cesium Chloride, Sodium Dihydrogen Phosphate Monohydrate, Sodium Phosphate Dibasic Dihydrate, Sucrose, DMSO-*d*₆, CDCl₃, carbonyl cyanide 4-(trifluoromethoxy) phenyl hydrazone (FCCP) were purchased from Sigma-Aldrich; HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) was bought from Fluka; Valinomycin was obtained from Alfa Aesar; Egg yolk L- α -phosphatidylcholine (EYPC), Brain PS were obtained from Avanti Polar Lipids.

Acetonitrile, ethyl acetate, chloroform, hexane and methanol were bought from VWR and used without further purification.

¹H-NMR spectra were recorded on a Bruker Avance III 400 MHz. BC-535A bilayer clamp (Warner Instrument Corp.) was used for recording all single channel currents. Perkin Elmer FL6500 was used for recording all the fluorescence spectra and Perkin Elmer UV/VIS lambda 365+ was used for UV studies. Stopped-flow instrument (SFM3000+ MOS450. Bio-Logic SAS, Claix, France) was used for evaluating water net permeability. ChemDraw was used to draw the chemical structure, experimental illustrations were drawn in biorender and graphs were plotted using excel and origin. For NMR analysis Mestrenova software was used.

2. Synthesis: Compounds **5**, **4**, **3** and **2** were prepared according to the procedure mentioned in the literature^{S1,S2}.



Scheme S1: Synthesis scheme for the synthesis of compound **1**.

NMR Data for compounds **5**, **4**, **3** and **2**:

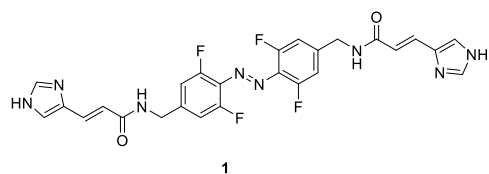
5: ¹H NMR (400 MHz, CDCl₃) δ 7.14 (dd, *J* = 6.0, 2.3 Hz, 2H), 4.27 (s, 2H). (NMR data was matched with reported data in literature.)^{S2}

4: ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.29 (t, *J* = 6.2 Hz, 1H), 6.75 (dd, *J* = 7.6, 2.2 Hz, 2H), 5.04 (s, 2H), 3.96 (d, *J* = 6.2 Hz, 2H), 1.38 (s, 9H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 155.86, 152.16, 149.79, 127.33, 123.92, 109.86, 78.02, 42.46, 28.31. ¹⁹F NMR (376 MHz, DMSO-*d*₆) δ -131.38.

3: ¹H NMR (400 MHz, CDCl₃) δ 6.98 (d, *J* = 9.6 Hz, 4H), 6.78 (d, *J* = 8.5 Hz, 2H), 5.00 (s, 2H), 4.92 (s, 1H), 4.35 (d, *J* = 6.4 Hz, 4H), 4.26 (d, *J* = 6.4 Hz, 2H), 1.47 (s, 18H), 1.45 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 157.19, 155.95, 154.61, 153.14, 150.61, 144.62, 130.67, 111.22, 111.01, 110.70, 110.48, 80.40, 44.01, 43.72, 28.48. ¹⁹F NMR (376 MHz, CDCl₃) δ -118.75, -120.10.

2: ¹H NMR (400 MHz, MeOD) δ 7.34 (d, *J* = 9.3 Hz, 4H), 7.16 (d, *J* = 7.8 Hz, 2H), 4.23 (s, 4H), 4.10 (s, 2H). ¹³C NMR (101 MHz, MeOD) δ 158.05, 155.45, 154.15, 151.62, 139.87, 139.77, 139.67, 132.75, 132.65, 132.56, 114.58, 114.35, 114.06, 43.21, 42.96. ¹⁹F NMR (376 MHz, MeOD) δ -76.97, -121.58.

The synthetic procedure compound 1: (E)-3-(1H-imidazol-4-yl) acrylic acid was dissolved in neat thionyl chloride and refluxed overnight at 78 °C. The solution was then evaporated the next day under rota evaporator to get the (E)-3-(1H-imidazol-4-yl) acrylic acid chloride. Sequentially, **2** (1 equiv) was dissolved in THF and DIPEA (di-isopropyl ethyl amine) (3 equiv) was added, and the solution was allowed to stir at room temperature for 30 minutes. (E)-3-(1H-imidazol-4-



yl) acrylic acid chloride was further dissolved in THF and was added dropwise to amine and DIPEA solution. The solution was allowed to stir under reflux condition overnight at 66 °C. The next day aliquot of the solution was given for liquid chromatography mass spectrometry (LCMS) analysis for the confirmation of the product. The purification of the crude solution was done through HPLC after the confirmation of the product through LCMS to obtain compound 1 [13mg, Yield = 5%]. ¹H NMR of the compound revealed a mixture of E and Z isomers in the final compound. The protons for both the isomers are marked in the NMR spectrum. (Figure S11 and S12) **¹H NMR 1-E (400 MHz, DMSO-*d*₆)** δ 8.94 (t, *J* = 5.9 Hz, 4H), 7.91 (s, 2H), 7.43 (d, *J* = 15.8 Hz, 2H), 7.25 (d, *J* = 10.4 Hz, 4H), 6.71 (d, *J* = 15.9 Hz, 2H), 4.49 (d, *J* = 5.9 Hz, 4H). **¹H NMR 1-Z (400 MHz, DMSO-*d*₆)** δ 8.89 (s, 2H), 8.83 (t, *J* = 6.0 Hz, 2H), 7.88 (s, 2H), 7.39 (d, *J* = 15.8 Hz, 2H), 7.09 (d, *J* = 9.0 Hz, 4H), 6.66 (d, *J* = 15.9 Hz, 2H), 4.39 (d, *J* = 6.0 Hz, 4H). **¹³C NMR (126 MHz, DMSO-*d*₆)** δ 164.71, 158.40, 158.14, 157.87, 155.75, 153.69, 151.70, 149.71, 146.28, 146.21, 144.31, 136.37, 136.34, 126.27, 122.76, 122.54, 120.43, 111.55, 111.53, 111.38, 111.05, 110.89, 41.81, 41.46. **¹⁹F NMR (376 MHz, DMSO-*d*₆)** δ -120.58, -121.18. **HRMS (ESI) m/z:** [M+2H]⁺ Calcd. for [C₂₆H₂₀F₄N₈O₂H₂⁺] 277.0896, Found 277.0897.

3. NMR and MS Spectrum

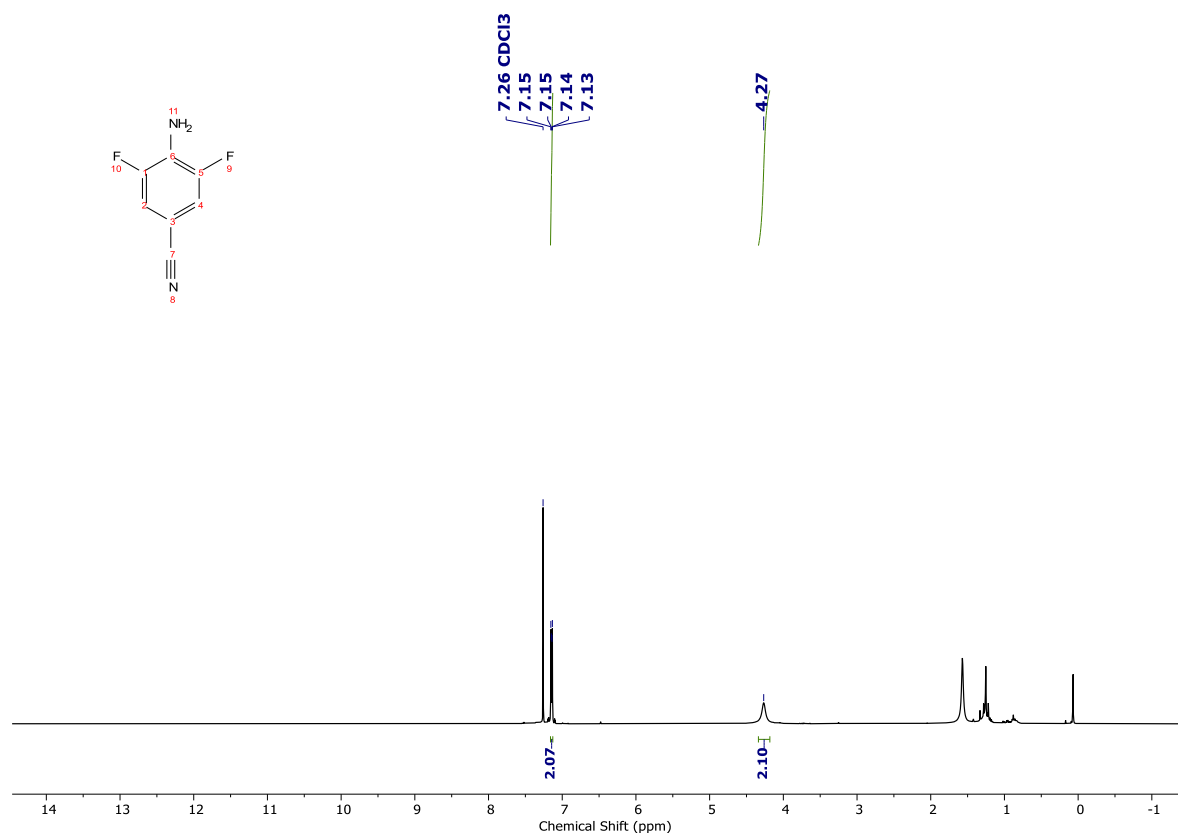


Figure S1: ¹H NMR (400 MHz) of **5** in CDCl₃.

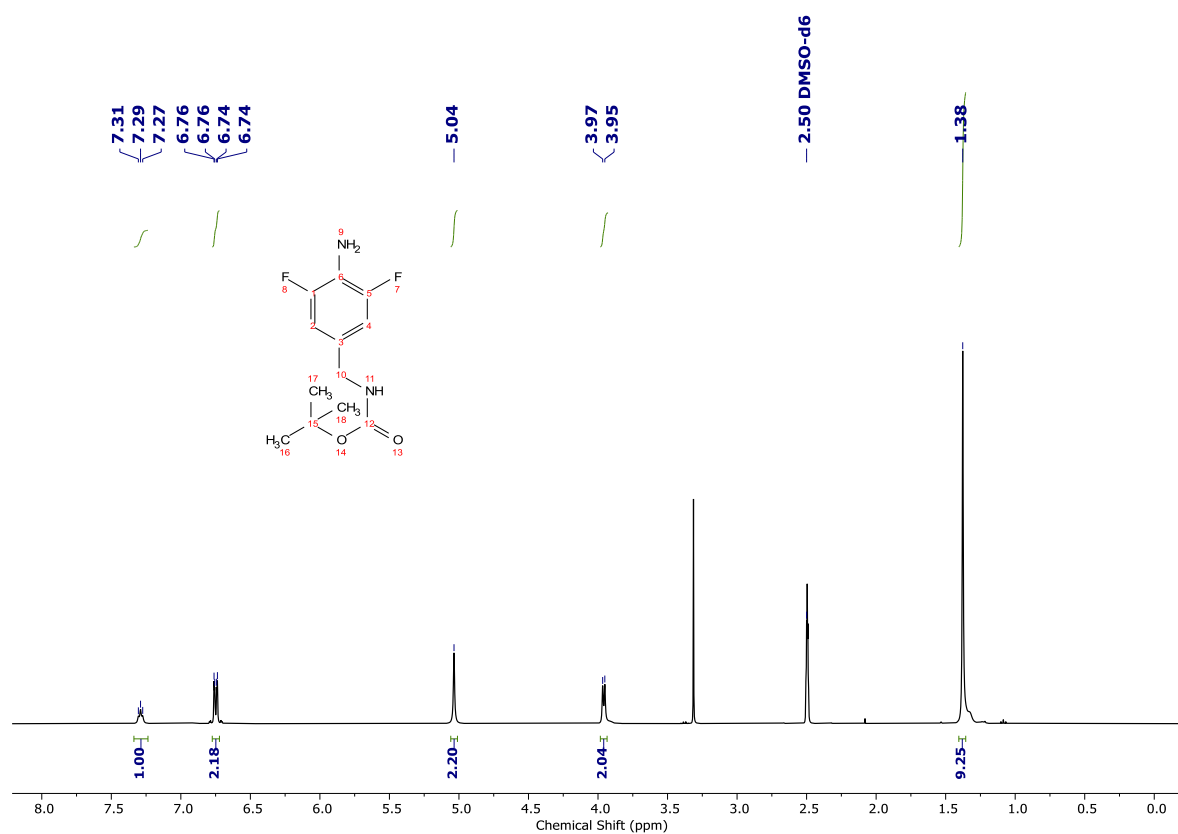


Figure S2: ¹H NMR (400 MHz) of **4** in DMSO-*d*₆.

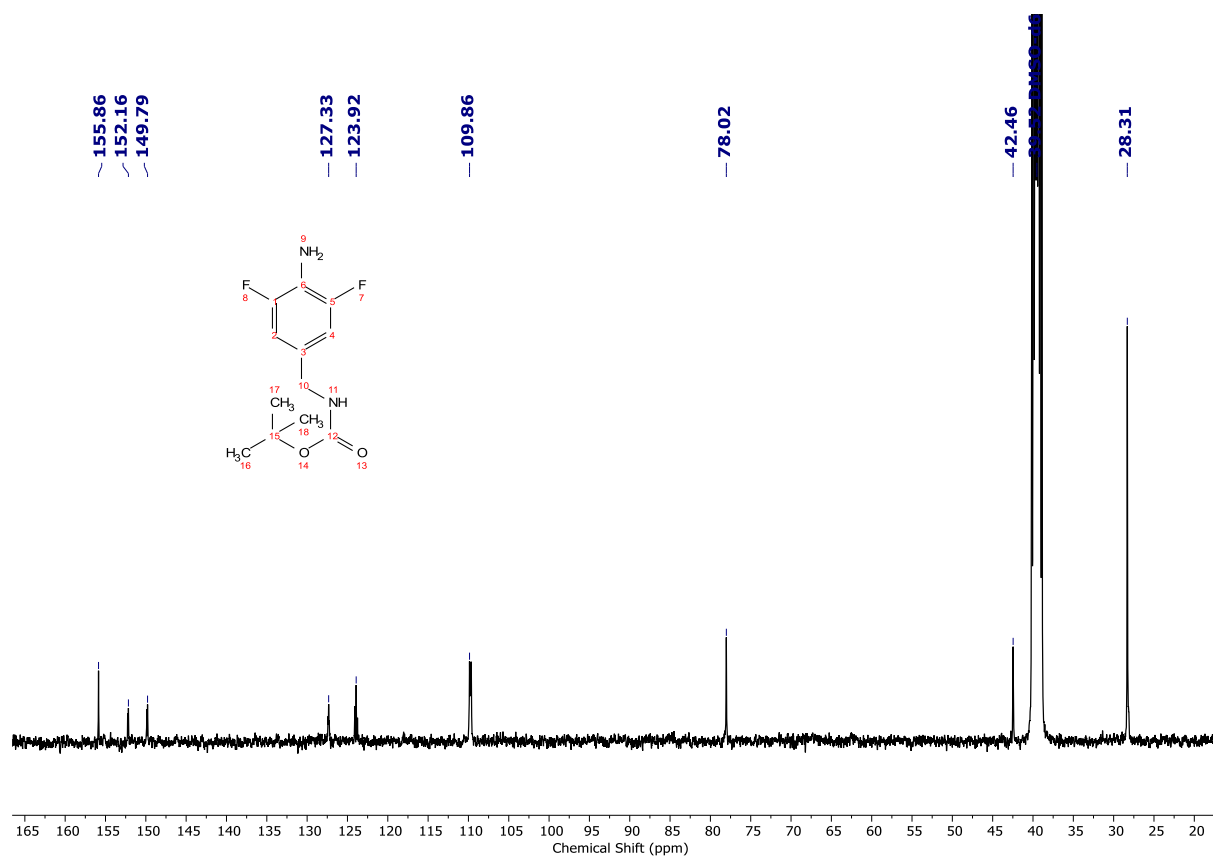


Figure S3: ¹³C NMR (101 MHz) of **4** in DMSO-*d*₆.

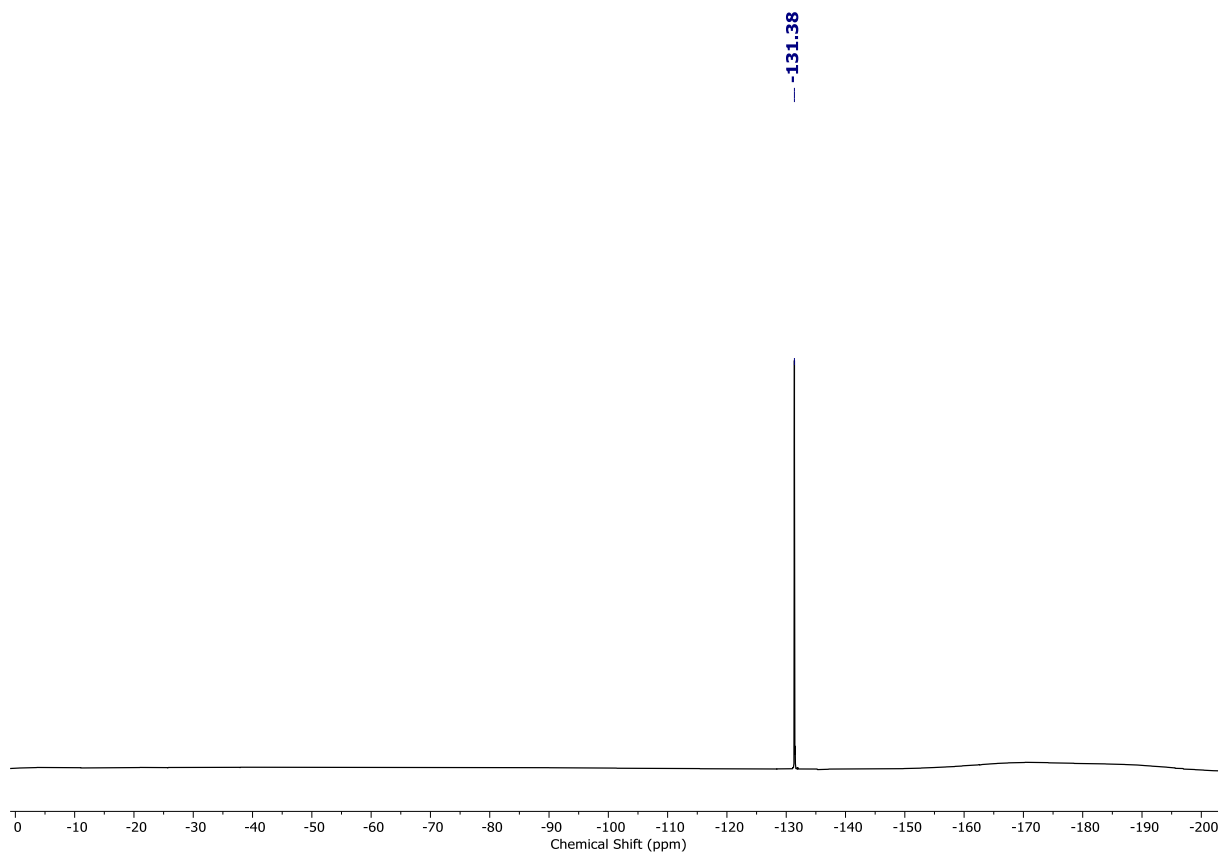


Figure S4: ^{19}F NMR (376 MHz) of compound **4** in $\text{DMSO-}d_6$.

PRW-15.10.fid
1H CDCl3 {D:\nsa\} nsa 34

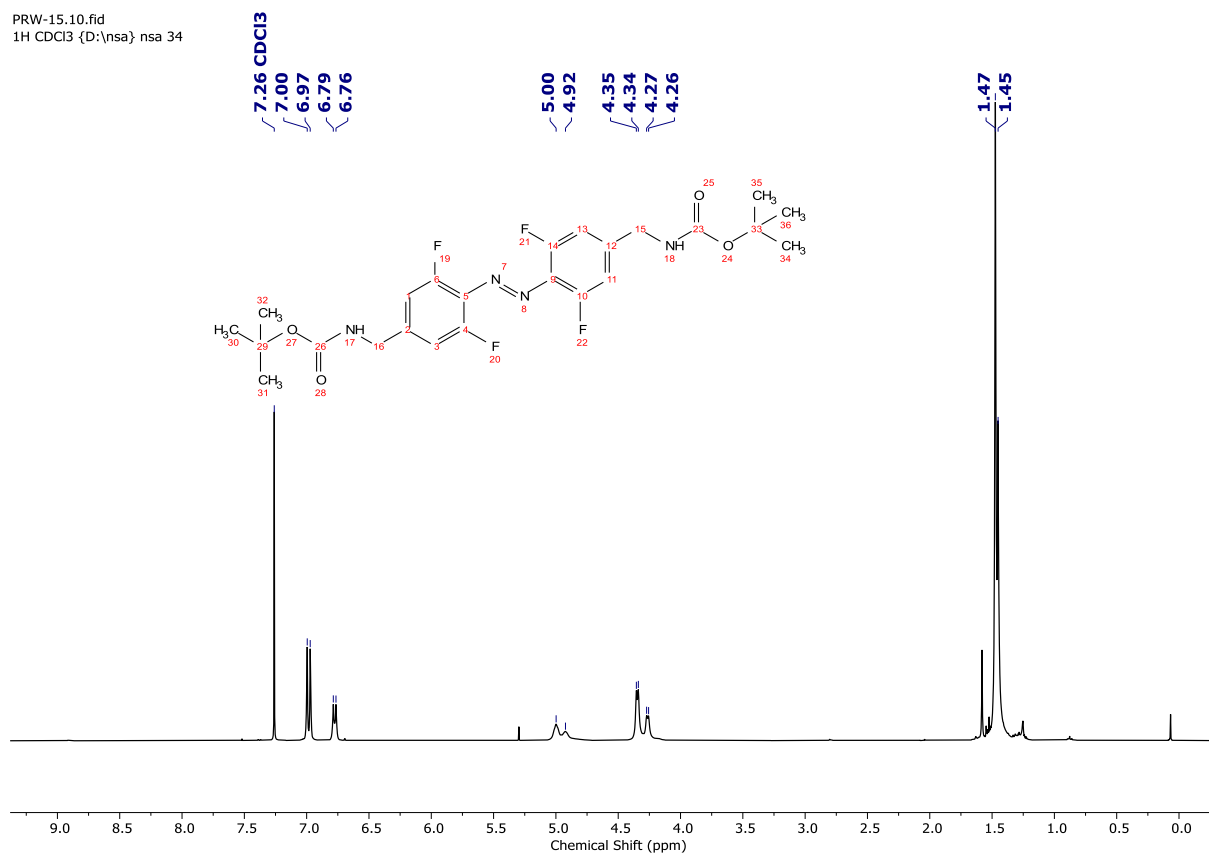


Figure S5: ^1H NMR (400 MHz) of **3** (E and Z mixture) in CDCl_3 .

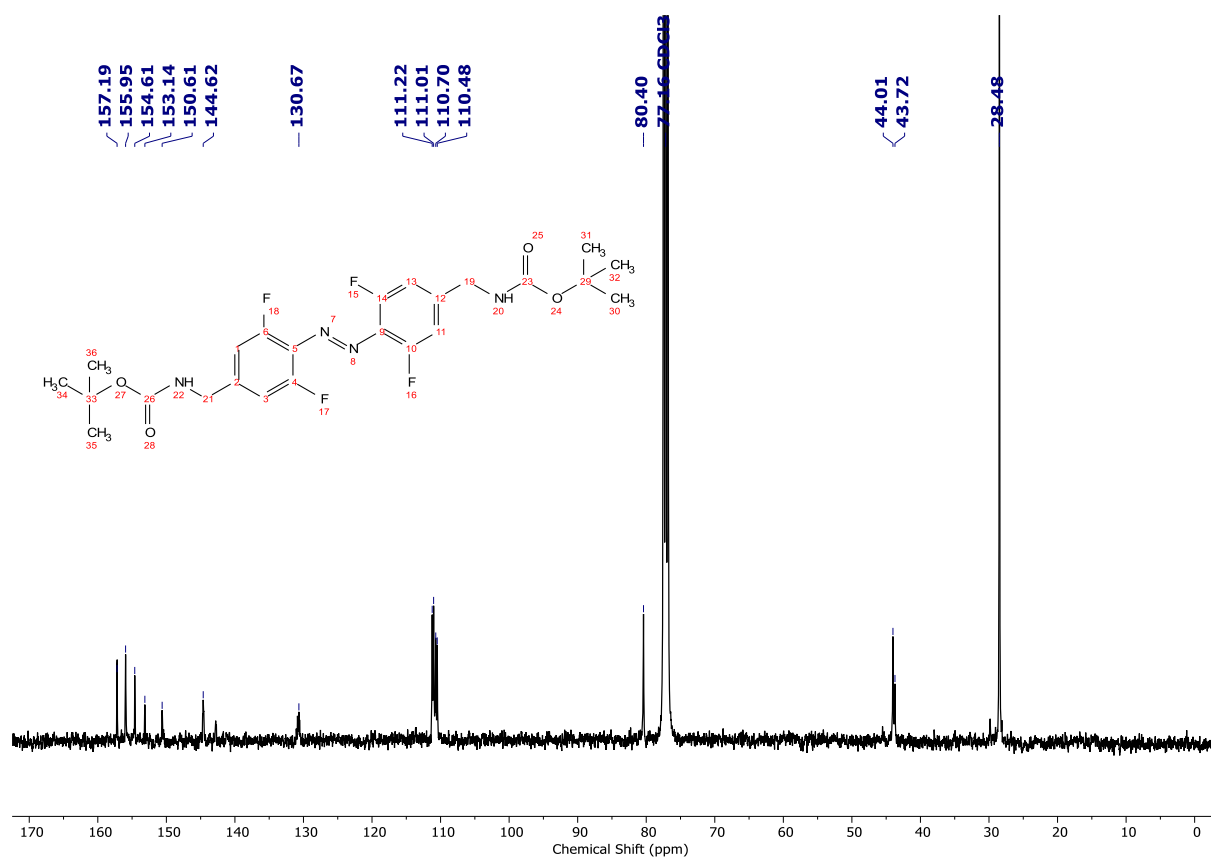


Figure S6: ¹³C NMR (101 MHz) of compound **3** in CDCl₃.

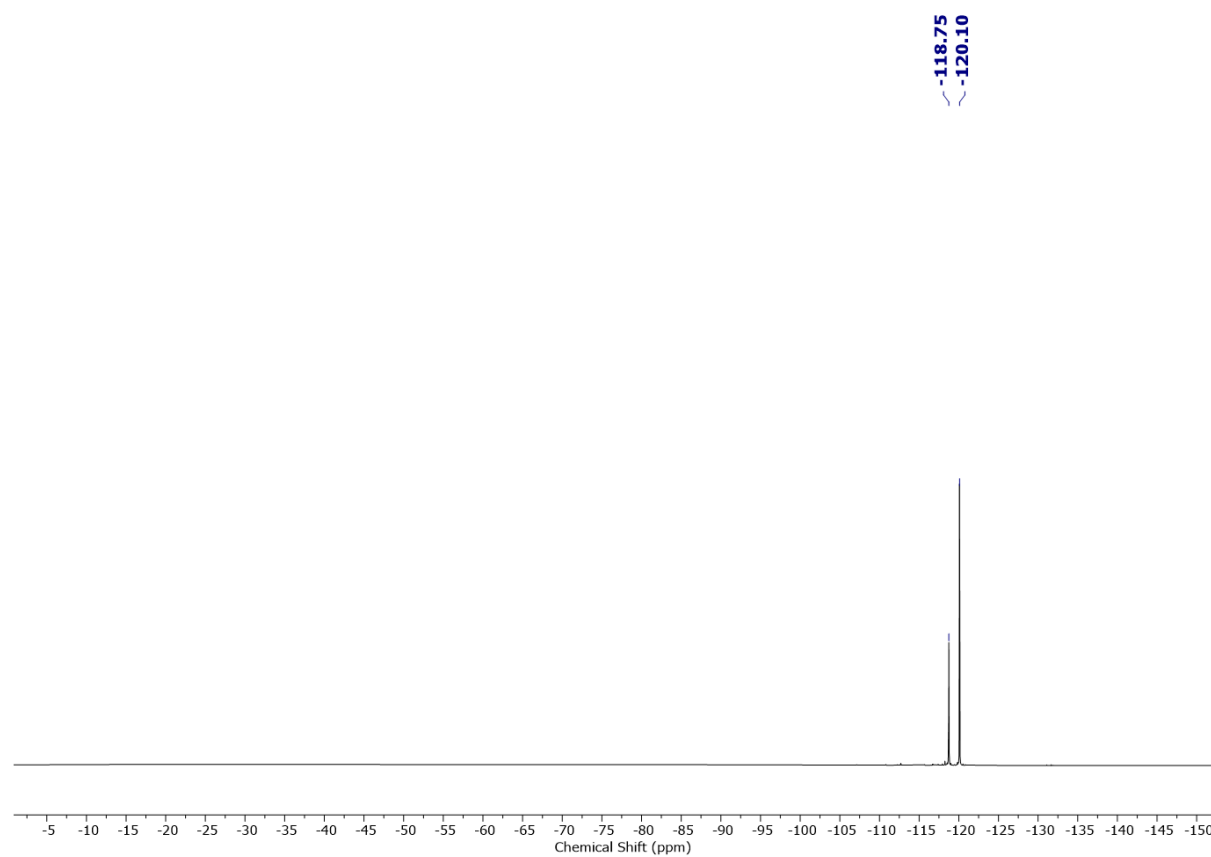


Figure S7: ¹⁹F NMR (376 MHz) of compound **3** in CDCl₃.

PRW-01-33.10.fid
1H MeOD {D:\nsa} nsa 15

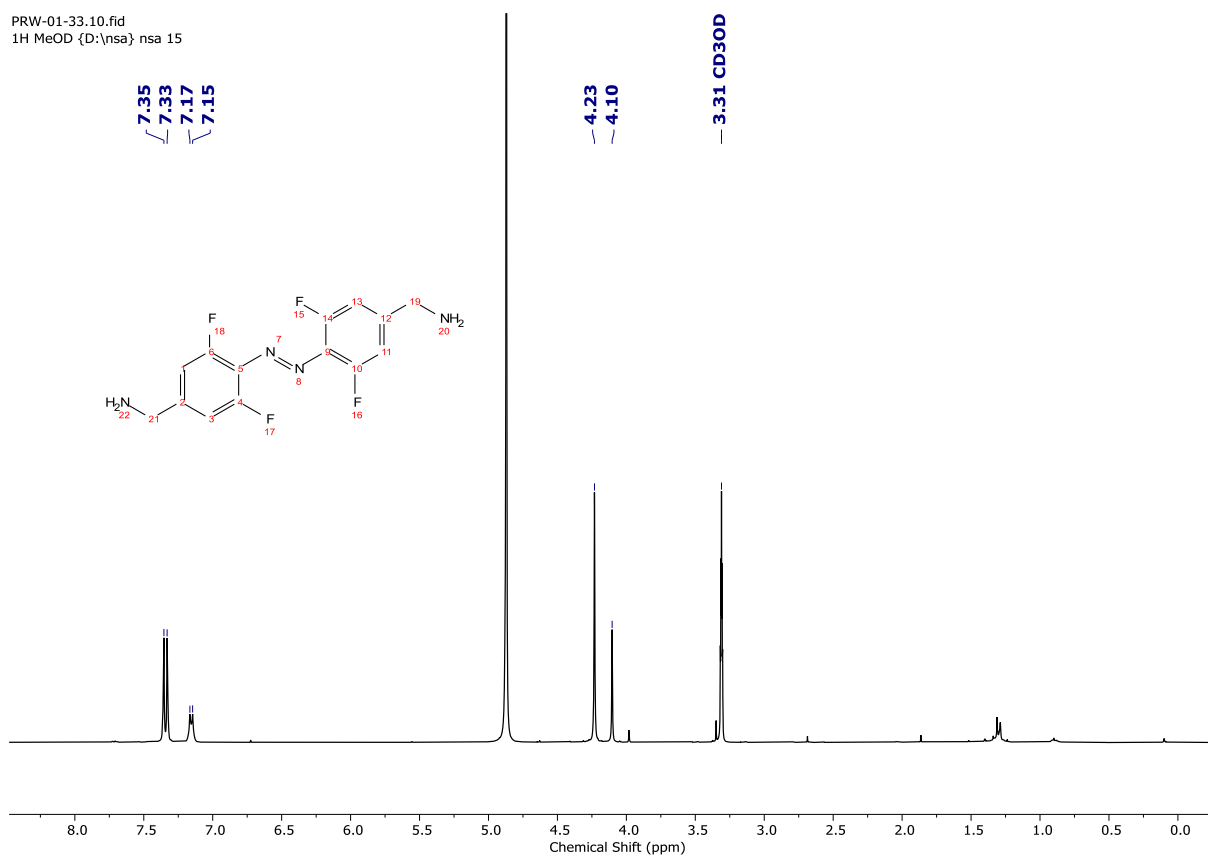


Figure S8: ¹H NMR (400 MHz) of **2** (E and Z mixture) in CD₃OD

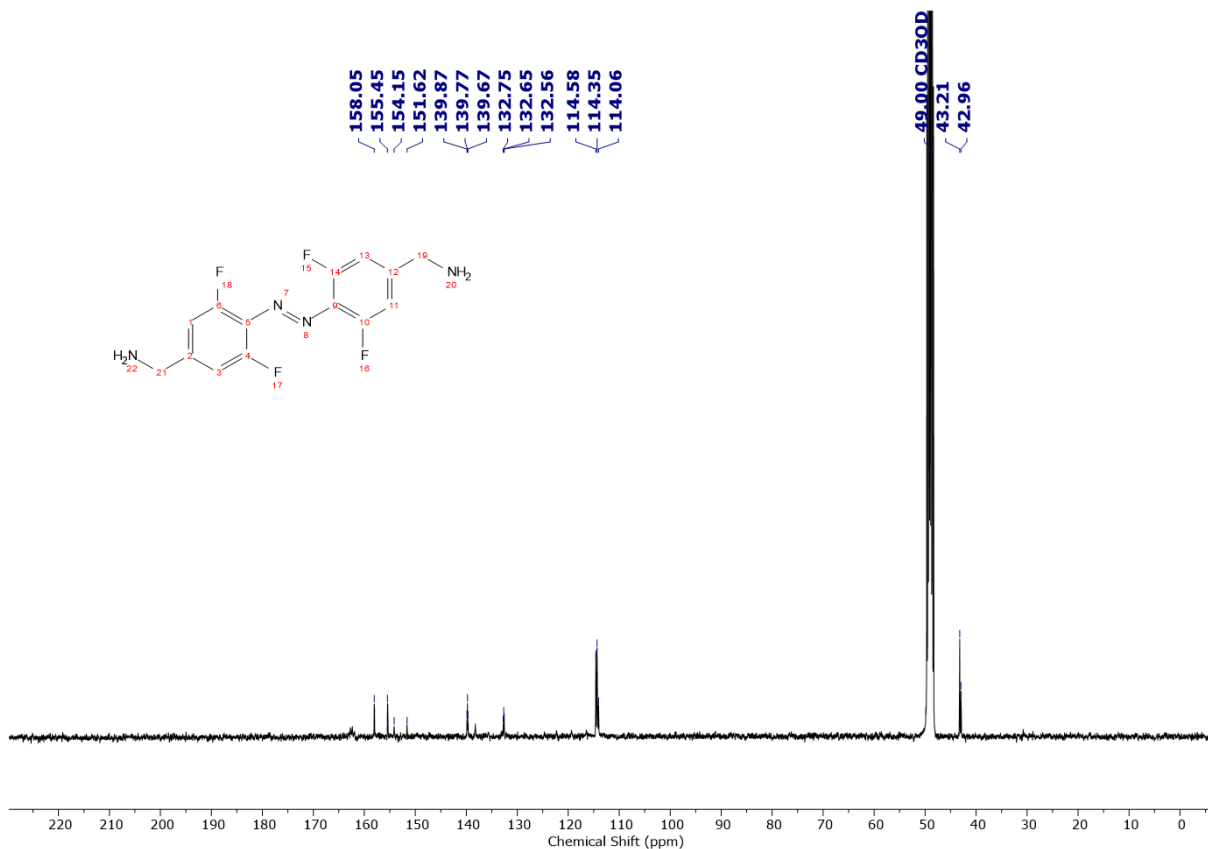


Figure S9: ¹³C NMR (101 MHz) of compound **2** in CD₃OD.

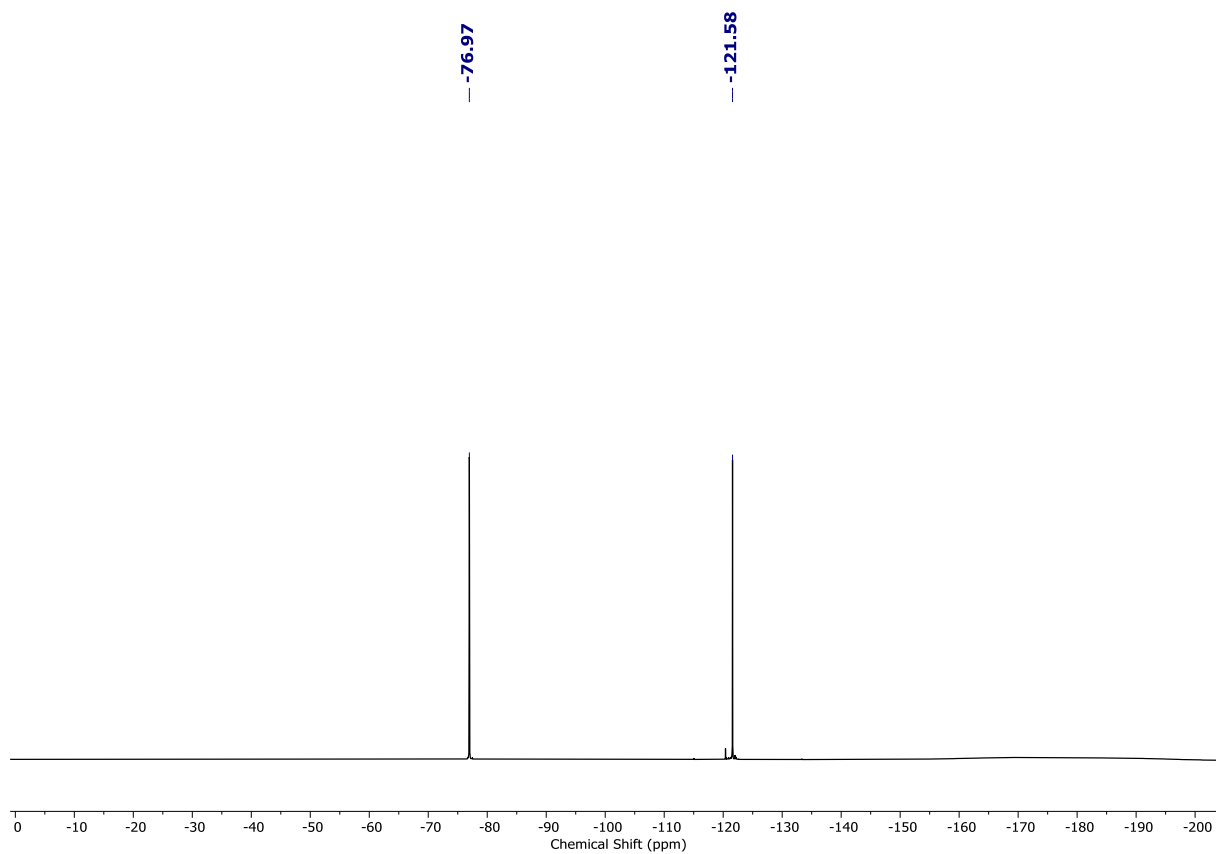


Figure S10: ^{19}F NMR (376 MHz) of compound **2** in CD_3OD .

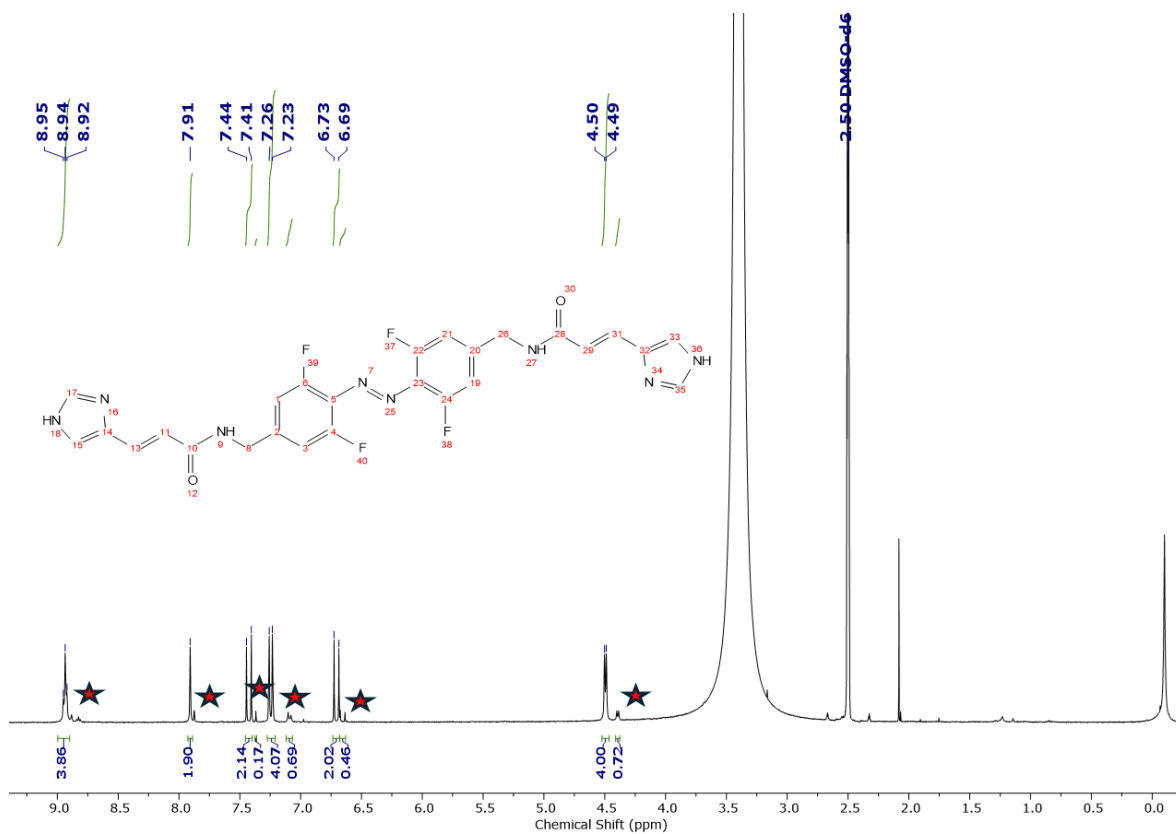


Figure S11: ^1H NMR (400 MHz) of **1-E** (84%) in $\text{DMSO}-d_6$, star represents protons for *Z* conformer.

PRW-38-505nm-Z-2hr.10.fid
1H DMSO {D:\nsa} nsa 57

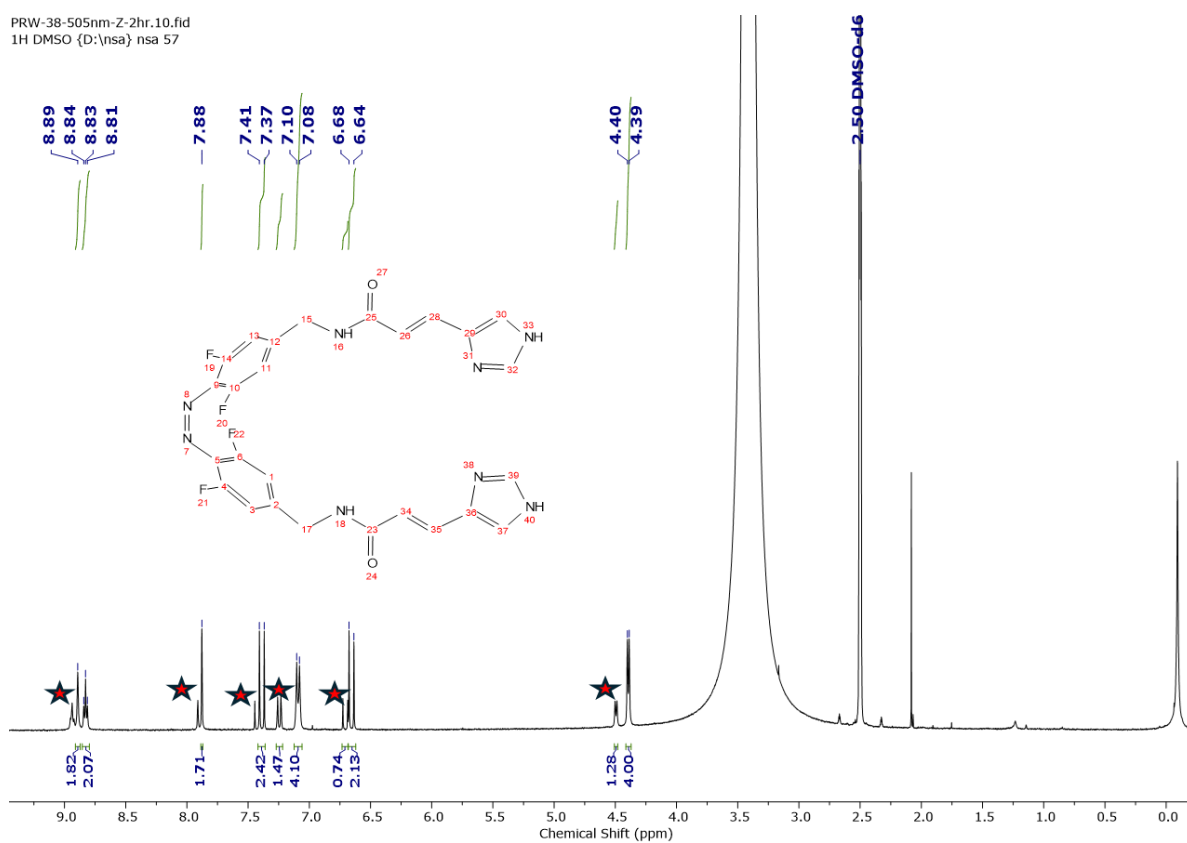


Figure S12: ^1H NMR (400 MHz) of 1-Z (75%) in $\text{DMSO}-d_6$, star represents protons for *E* conformer

WAN-13.100051.fid

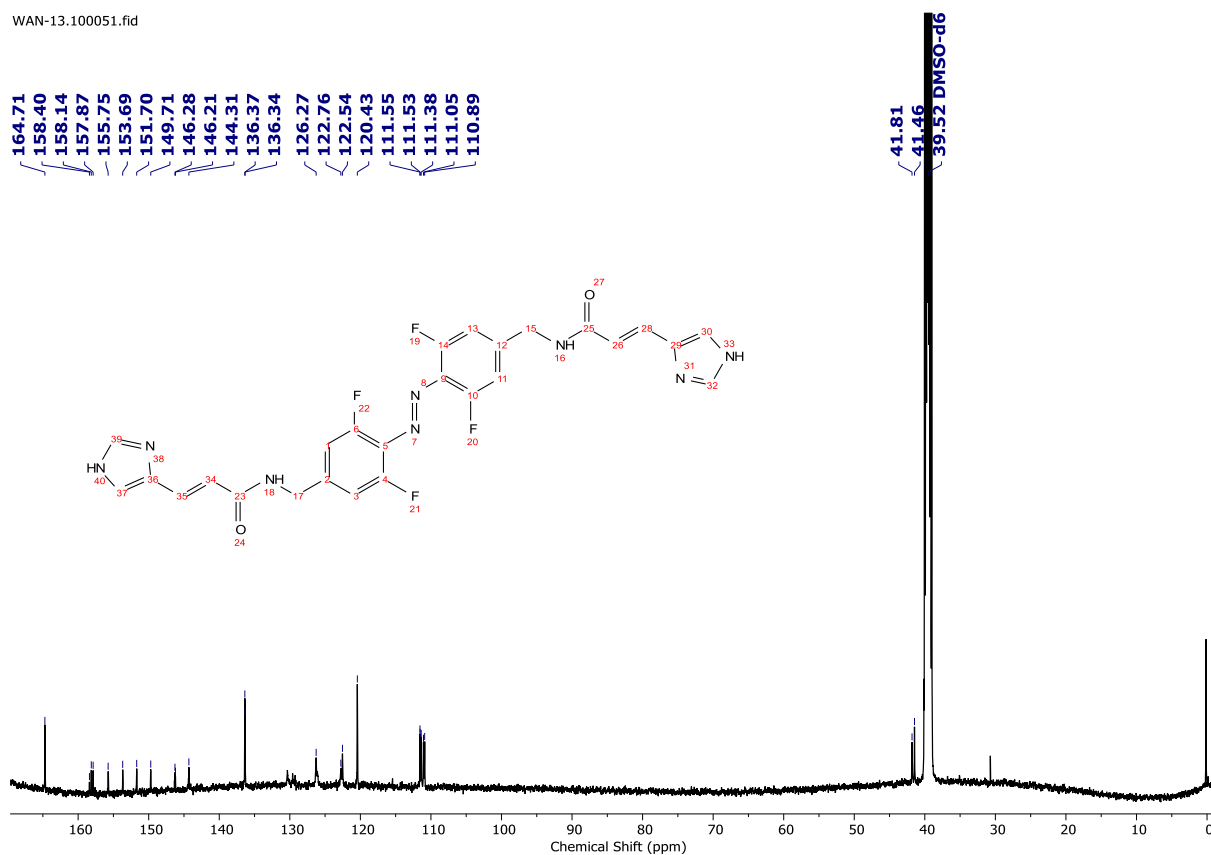


Figure S13: ^{13}C NMR (126 MHz) for compound 1 in $\text{DMSO}-d_6$.

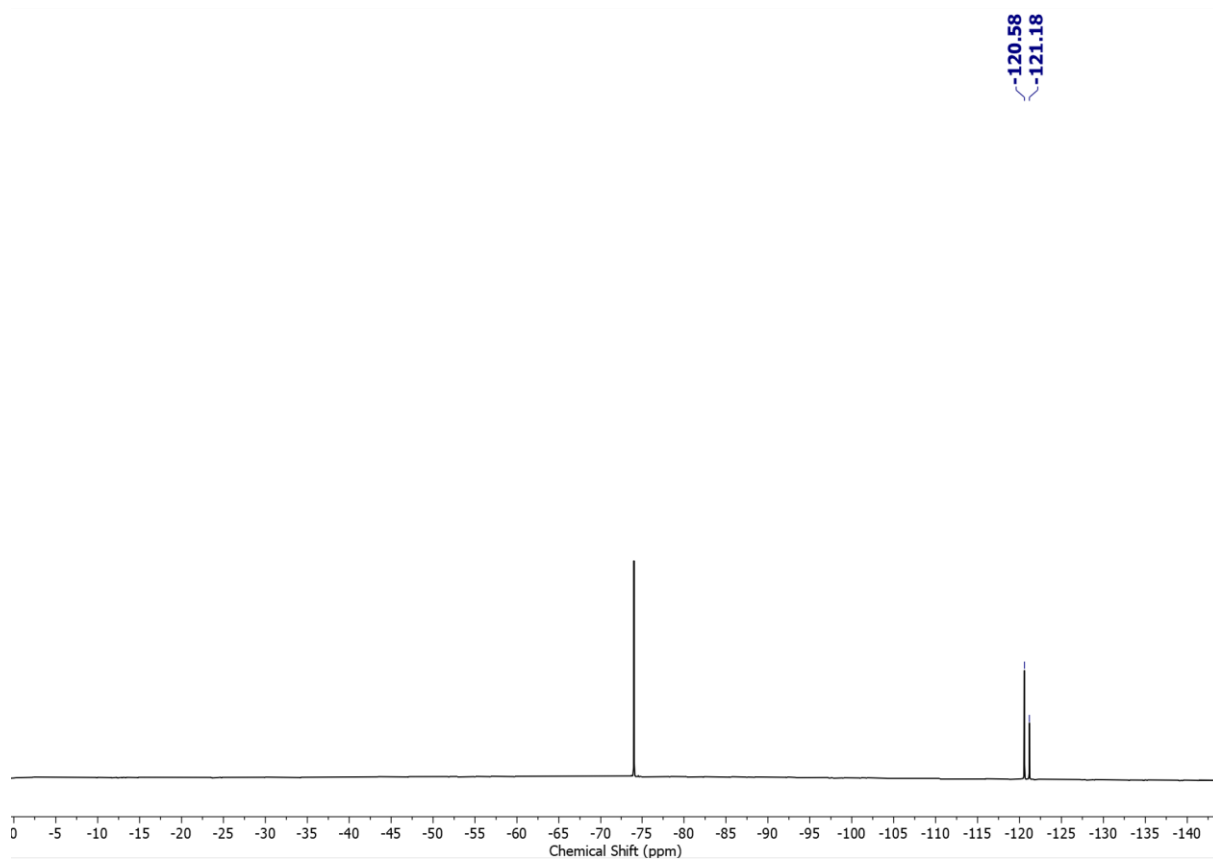


Figure S14: ¹⁹F NMR (376 MHz) for **1** in DMSO-*d*₆.

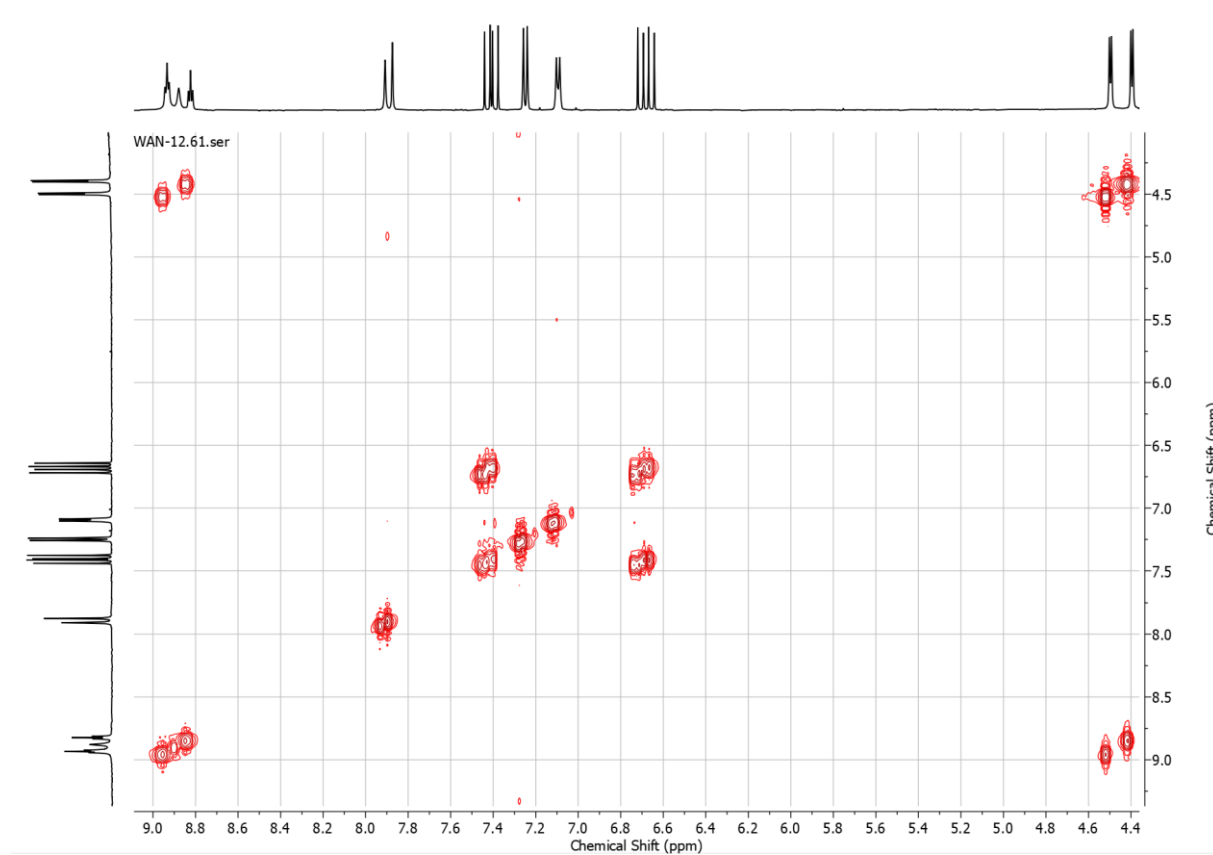


Figure S15: COSY ¹H—¹H coupling for compound **1**.

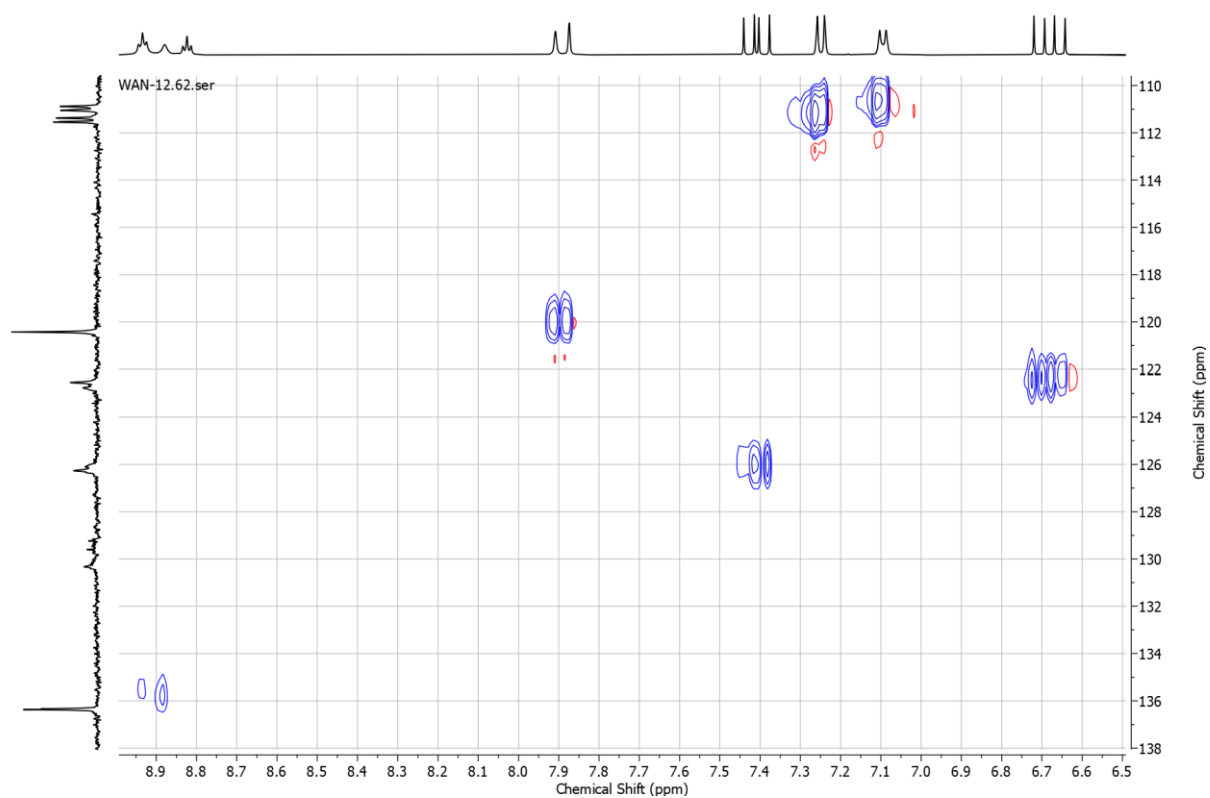


Figure S16: HSQC of **1**, showing $^1\text{H} - ^{13}\text{C}$ correlation.

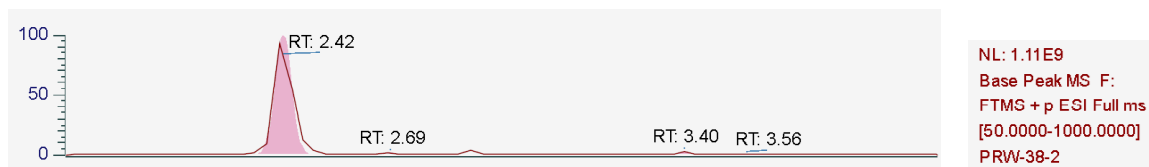


Figure S17: LCMS trace for compound **1** with retention time of 2.42 (pink peak).

PRW-38-2 #118-126 RT: 2.43-2.54 AV: 4 SB: 34 1.97-2.22, 3.95-4.68 NL: 4.01E8
T: FTMS + p ESI Full ms [50.0000-1000.0000]

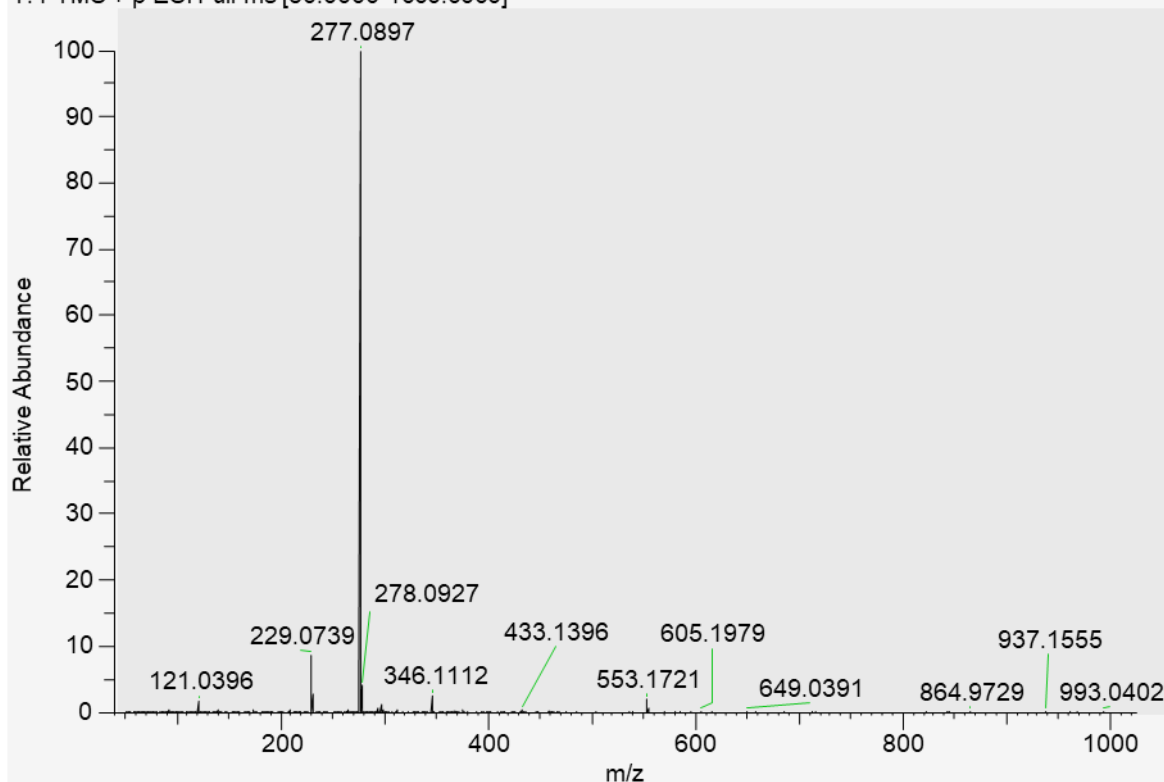


Figure S18: HRMS for compound 1 showing peak for $[M+2H]^+$ Calculated - $[C_{26}H_{20}F_4N_8O_2H_2^+]$ 277.0896, Found 277.0897.

4. Photoswitching Studies: Photoirradiation setup was prepared using the Thorlabs equipments and setup was the same used in the literature with M405L4 and M505L4 LEDs.^{S2}

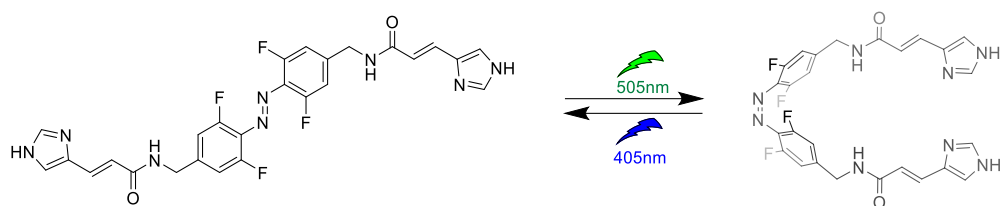


Figure S19: Representation of photoswitching in between the isomers for compound **1**.

UV-Vis Studies:

Photoswitching studies for **1** was done in 2 systems first is in DMSO solution and second with the vesicles

For vesicle solution, the vesicles were prepared as same for water transport studies with egg yolk phosphatidylcholine, phosphatidylserine and cholesterol in the ratio of (4:1:5). **1** (20 μ M) was dissolved in 1.88 mL of PBS buffer solution with 100 μ L of the prepared vesicles. Initially, a spectrum was recorded for a mixture of E and Z isomers (after synthesis) after which the same sample was irradiated with 505 nm wavelength LED until there was no further change in the spectrum. Furthermore, subsequent irradiation of the same sample was done by 405 nm LED till the PSS was reached again. This process was repeated for 5 cycles ensuring that the photostationary state is achieved after each of the irradiation. Finally, the spectrum was plotted using Origin software and Absorbance at 330 nm and 407 nm (figure S20) was monitored to check the conversion of isomers after each irradiation.

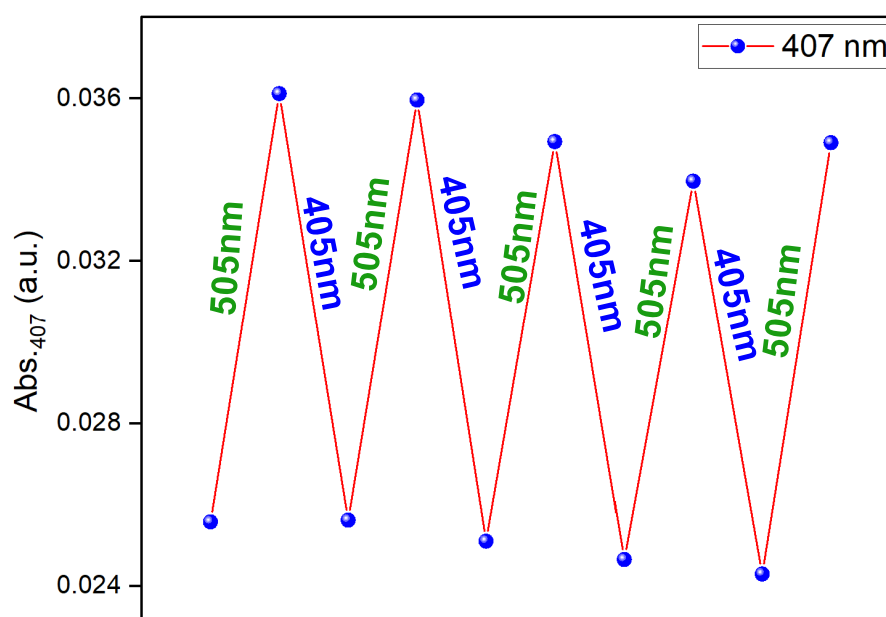


Figure S20: Absorbance changes at 407 nm after each irradiation for various cycles of compound **1** with the vesicles.

Similar procedure was also followed in DMSO solution, where 1 (64 μM) was dissolved in DMSO and similar procedure as before was followed to achieve the UV-Vis spectra as observed in figure S21. Further, the absorbance values at 323 nm (figure S22A) and 421 nm (figure S22B) were observed to verify the efficiency of the photoswitch.

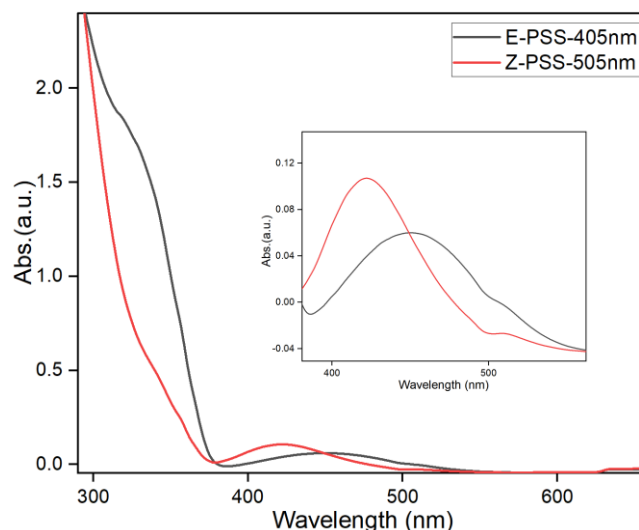


Figure S21: UV- Vis spectrum showing conversion between 1-*E* and 1-*Z* isomers in DMSO.

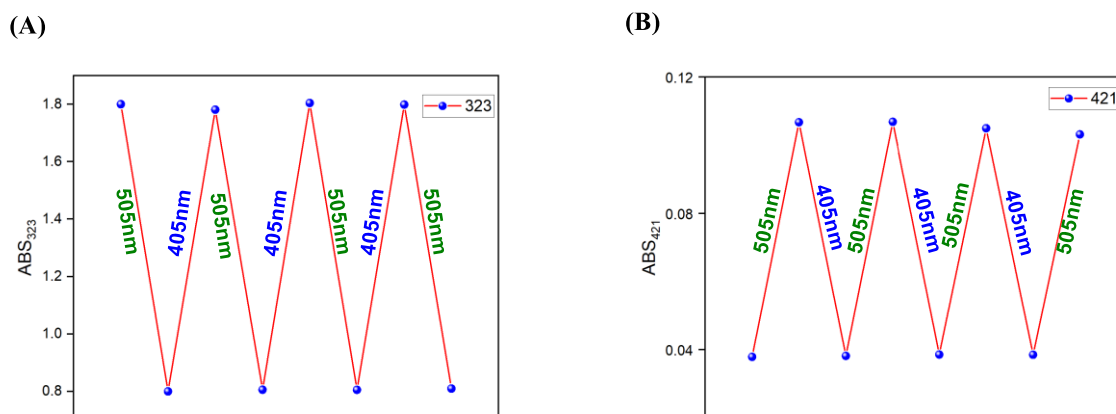


Figure S22: Absorbance changes at 323nm (A) and 421nm (B) after each irradiation for various cycles.

^1H NMR Studies: To determine the percentage of each isomer at the PSS, NMR spectra were recorded after each irradiation step until equilibrium was reached. Compound 1 was dissolved in DMSO- d_6 , and an initial NMR spectrum (maroon) was recorded before irradiation, as shown in Figure S23. The sample was then irradiated with a 405 nm LED, converting most of the compound to the *E* isomer. Once the PSS was reached, the integration of peaks corresponding to the *E* and *Z* isomers was performed separately. The ratio of these integration values was used to determine the *E/Z* ratio at the PSS. This process was repeated for multiple isomeric peaks at different chemical shifts. The average percentage of the *E* isomer at 405 nm PSS was found to be 84%. Similarly, after irradiation with a 505 nm LED, the percentage of the *Z* isomer at the PSS was determined to be 75% (Figure S23).

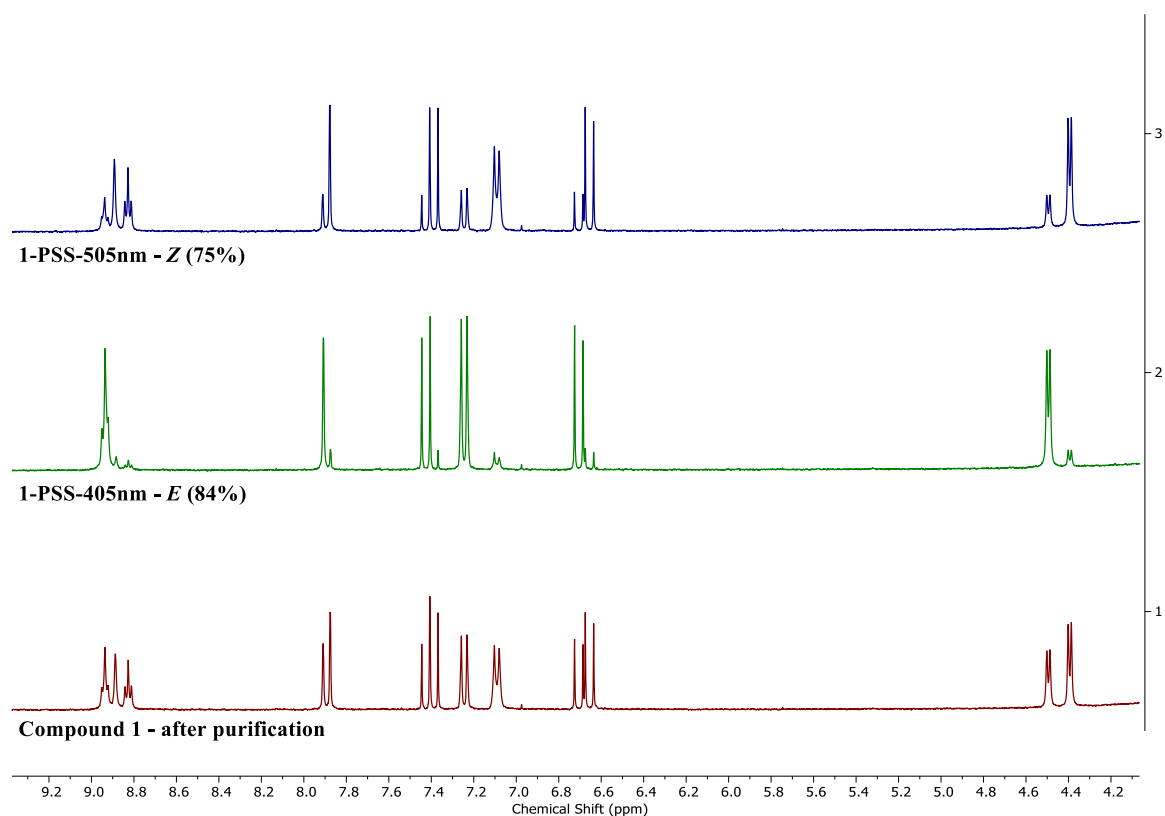


Figure S23: ^1H NMR photoirradiation study indicating the *E* and *Z* conformers of **1**.

5. Ion Transport Studies

Preparation of Sodium Phosphate Buffer Salt and Compound stock solutions: A 10 mM sodium phosphate buffer (pH 6.4) was prepared by dissolving 1.028 g of sodium dihydrogen phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) and 0.454 g of sodium phosphate dibasic dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$) in 1 L of Milli-Q water. For each experiment, the necessary salts were dissolved in Milli-Q water as required. All stock solutions of the compounds were prepared in DMSO with serial dilution.

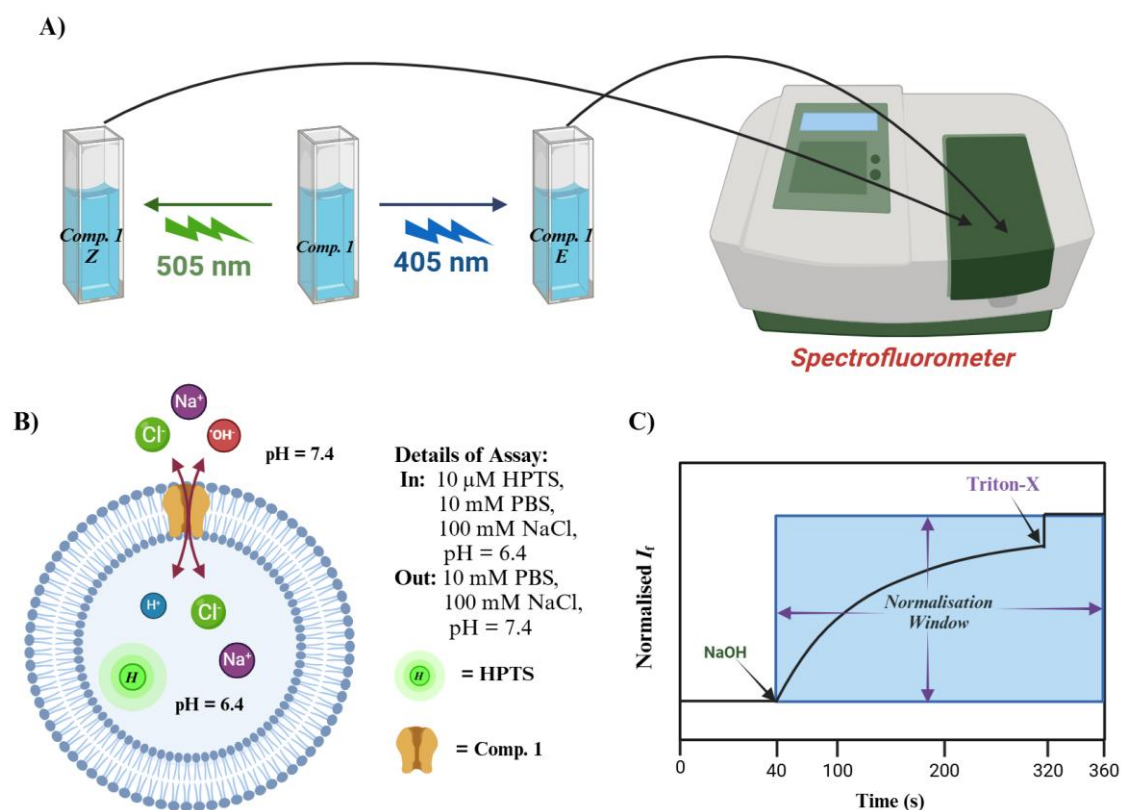


Figure S24: Experimental procedure followed during the fluorescence studies where irradiation of the sample with vesicles was done before each recording with different wavelengths of light (A). Illustration of HPTS assay with EYPC-LUVs \Rightarrow HPTS with NaCl (B) and the fluorescence spectra obtained during the assay highlighting the normalisation window (C).

Preparation of EYPC-LUVs \Rightarrow HPTS with NaCl: Large unilamellar vesicles (LUVs) were prepared using egg yolk L- α -phosphatidylcholine (EYPC) from a chloroform solution (2 mL, 50 mg). The solvent was slowly evaporated under vacuum at room temperature, followed by drying under high vacuum for two hours. The resulting thin lipid film was hydrated with 1 mL of buffer (10 mM sodium phosphate, pH 6.4, 100 mM NaCl) containing 10 μ M HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) for 40 minutes. The hydrated suspension underwent 5–10 freeze-thaw cycles (using liquid nitrogen and water at 30°C) to enhance vesicle formation. The resulting white suspension was then extruded 21 times through a 100 nm polycarbonate membrane, converting large multilamellar vesicles (LMVs) into LUVs with an average diameter of 100 nm. To remove excess HPTS dye from the extravesicular space, the LUV suspension was purified using size exclusion chromatography (SEC) with Sephadex G-50 as the stationary phase and phosphate buffer (100 mM NaCl) as the mobile phase. The purified LUV suspension was then diluted with the mobile phase to obtain 7 mL of an 11 mM lipid stock solution, assuming full lipid incorporation.

Concentration dependent activity for 1: A 100 μ L aliquot of the stock vesicle solution was diluted in 1.85 mL of the corresponding buffer (10 mM PBS, pH 6.4, with 100 mM NaCl). **1** was added with increasing concentration and irradiated with 405 nm (for E) and 505 nm (for Z) and then transferred into

the quartz fluorometric cuvette (figure S24A). 405 nm irradiation was done before adding the vesicles to avoid excitation of HPTS dye while the 505nm irradiation was done with vesicles. The emission of HPTS at 510 nm was recorded at two excitation wavelengths (403 and 460 nm) simultaneously. At 40 s, 29 μ L of 0.5 M aqueous NaOH was added to the cuvette, leading to an approximate one-unit increase in extravesicular pH. Finally, at $t = 320$ s, the vesicles were lysed using 40 μ L of 5% aqueous Triton X-100 to eliminate the pH gradient between intra- and extravesicular compartments. A schematic representation of the vesicles during the experiment is shown in Figure S25B, along with the fluorescence kinetics observed during the assay (figure S24C). After normalising the fractional fluorescence intensity using equation S1 the graphs for E and Z are shown in figure S25.

$$I_F = [(I_t - I_0) / (I_\infty - I_0)] \quad \text{Equation S1}$$

Where I_0 is the fluorescence intensity before addition of 0.5 M NaOH, I_∞ is the final fluorescence intensity after addition of Triton-X, I_t is the fluorescence intensity at time t and I_F is the final normalised intensity at a given time t .

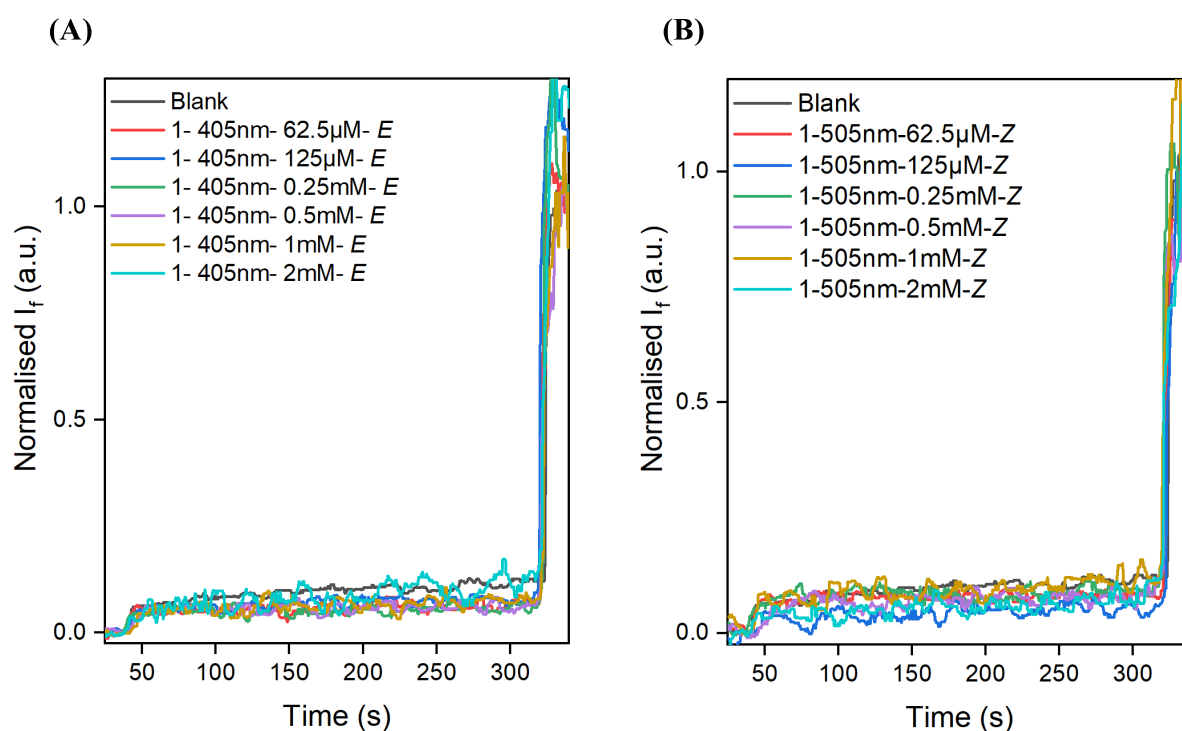
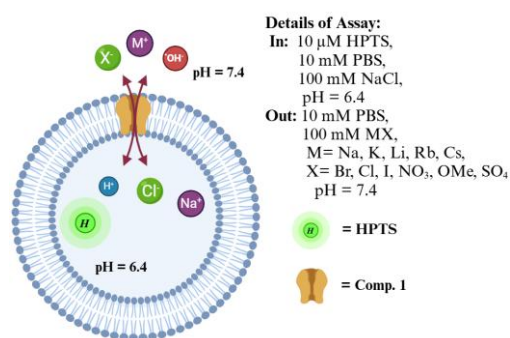


Figure S25: Ion transport activity for 1-E (A) and 1-Z (B) with NaCl salt across EYPC-LUVs \Rightarrow HPTS.

Salt Rejection Assay: A 100 μL aliquot of the stock vesicle solution was dissolved in 1.85 mL of the

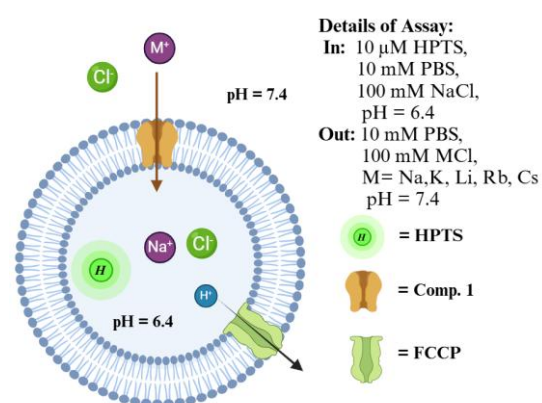


corresponding buffer (10 mM PBS, pH 6.4, with different salts). 1mM concentration of **1** was added, followed by irradiation at 405 nm (for E) and 505 nm (for Z), before being transferred into the spectrofluorometer. The emission of HPTS at 510 nm was simultaneously measured at two excitation wavelengths (403 and 460 nm). At $t = 40$ s, 29 μL of 0.5 M aqueous NaOH was introduced into the

cuvette, resulting in an approximate one-unit increase in extravesicular pH. Finally, at $t = 320$ s, 40 μL of 5% aqueous Triton X-100 was added to lyse the vesicles, eliminating the pH gradient between intra- and extravesicular compartments. The process was repeated for salts such as KCl, LiCl, RbCl, CsCl, NaBr, NaI, NaNO₃, NaOMe and Na₂SO₄. A schematic representation of the vesicle during the experimental process is depicted above and the obtained graphs were plotted and normalised using equation S1 for E and Z isomer.

Protonophore Coupled Assay:

FCCP Assay: In a quartz fluorometric cuvette, 100 μL of the stock vesicle solution was diluted in 1.85



mL of the corresponding buffer (10 mM PBS, pH 6.4, containing 100 mM MCl). 1mM concentration of **1** was added, followed by irradiation at 405 nm (for E) and 505 nm (for Z), before being transferred into the spectrofluorometer. The emission of HPTS at 510 nm was continuously monitored at two excitation wavelengths (403 and 460 nm) simultaneously. At $t = 20$ s, 20 μL of 50 μM carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) was

added, followed by 29 μL of 0.5 M aqueous NaOH at $t = 40$ s, inducing a pH gradient of approximately one unit across the vesicle membrane. Finally, at $t = 320$ s, the vesicles were lysed using 40 μL of 5% aqueous Triton X-100, effectively disrupting the established pH imbalance. A schematic representation of the vesicle during the experimental process is depicted above, while the fluorescence kinetics data obtained for different cationic salts (NaCl, KCl, LiCl, RbCl, CsCl), normalized for is presented in Figure S26.

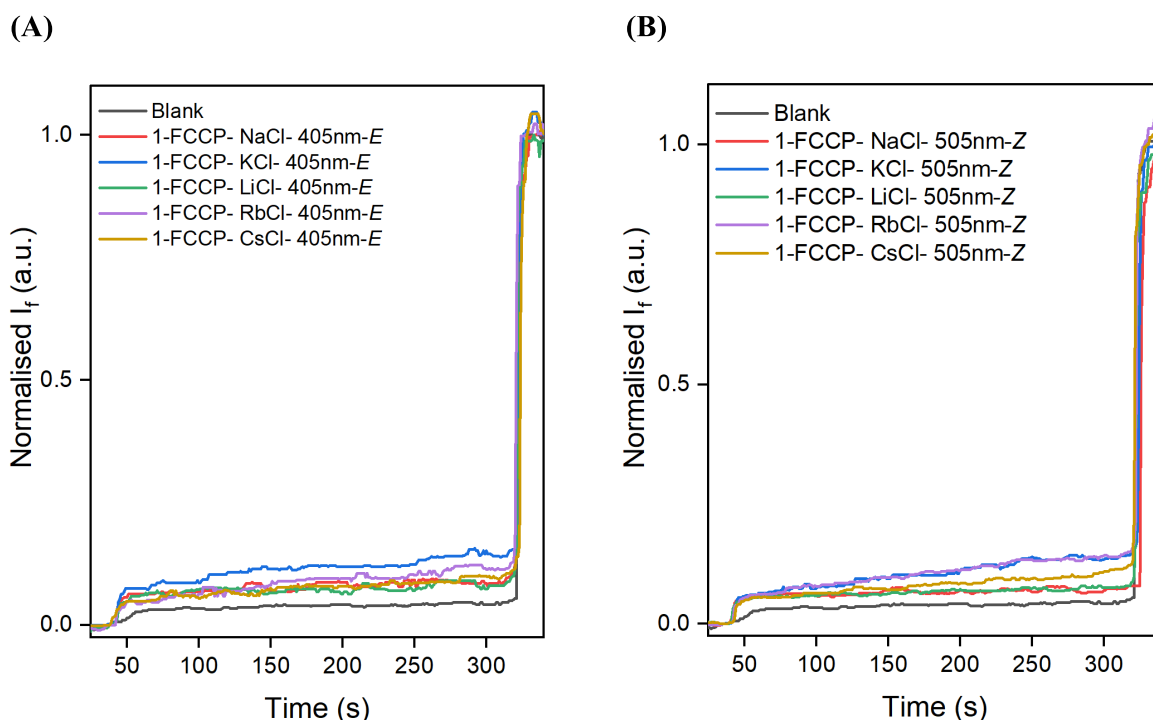
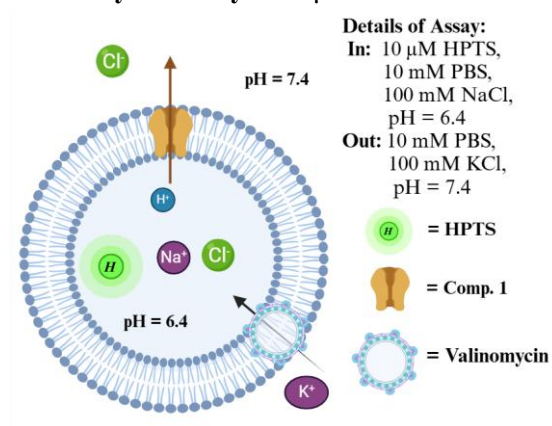


Figure S26: Ion transport activity of **1** in presence of FCCP (50 μ M) proton transporter for **1-E** (A) and **1-Z** (B).

Cationophore Coupled Assay:

Valinomycin Assay: 100 μ L of stock vesicle solution was suspended in 1.85 mL of 10 mM PBS pH 6.4



containing 100 mM of KCl and placed into a quartz fluorometric cuvette. Increasing concentration of **1** was added, followed by irradiation at 405 nm (for E) and 505 nm (for Z), before being transferred into the spectrofluorometer. The emission of HPTS at 510 nm was monitored at two excitation wavelengths (403 and 460 nm) simultaneously. At $t = 20$ sec valinomycin (25 μ M) was added and then at $t = 40$ sec 29 μ L of aqueous NaOH (0.5 M) was injected. Finally, at $t = 320$ s, the

vesicles are lysed with detergent (40 μ L of 5% aqueous Triton X-100), to disrupt the pH imbalance among the intravesicular and extravesicular solutions. The cartoon representation of the vesicle during the experiment is shown above and the obtained normalized result for both isomers are shown in manuscript (figure 3A and 3C). A gradual increase in transport activity was observed till 5mM in the case of Z isomer. So, we calculated the half maximal concentration value (EC₅₀) for Z isomer using hill equation with values at $t=310$ s (equation S2) (figure S27).

$$y = y_0 + (y_1 - y_0) * x^n / (k^n + x^n) \quad \text{Equation S2}$$

Where y_0 is the fluorescence activity of the blank i.e. without the addition of compound, y_1 is the maximum activity achieved by the compound, x is the concentration of the molecule inside the cuvette, k is the half maximal concentration (EC_{50}).

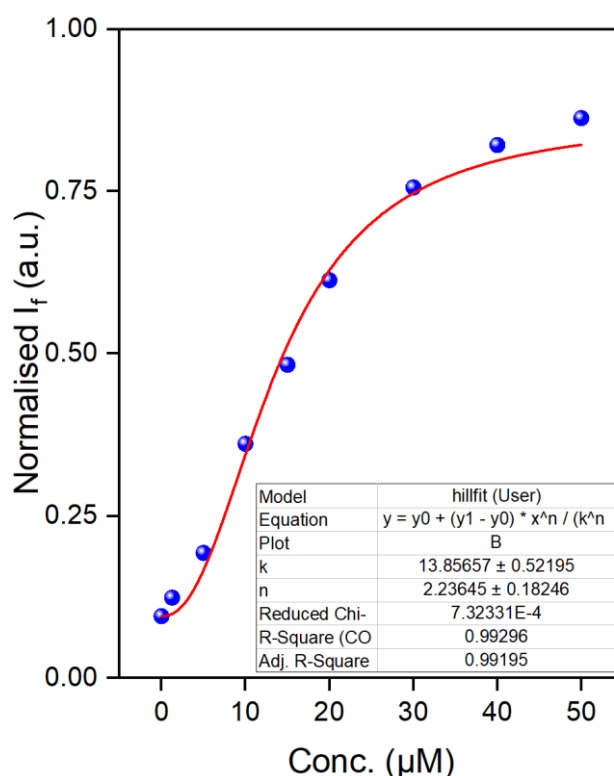


Figure S27: Hill plot for EC_{50} value determination with fluorescence intensity at $t = 310$ s for 1-Z.

Water Transport Assay:

LUVs Preparation: The liposomes were prepared using the film rehydration technique. A lipid mixture of egg yolk L- α -phosphatidylcholine (EYPC), L- α -phosphatidylserine (PS), and cholesterol (Chl) in a molar ratio of 4:1:5 was dissolved in a chloroform/methanol (1:1, v/v) solution. The organic solvent was evaporated using a rotary evaporator, and the resulting lipid film was further dried under vacuum in a desiccator for 3 hours. The dried film was rehydrated by vortex mixing with 1 mL of buffer (200 mM sucrose, 10 mM PBS, pH 6.4) at 25 °C for 1 hour, forming a milky suspension. This suspension underwent 10 freeze-thaw cycles using liquid nitrogen and a warm water bath of 30 °C. It was then extruded 21 times through a 100 nm polyethersulfone membrane (Whatman, UK; pore size: 0.1 μ m) to obtain monodisperse unilamellar vesicles. The final 1 mL suspension was diluted to 7 mL with the same buffer, achieving a lipid concentration of 8.61 mM.

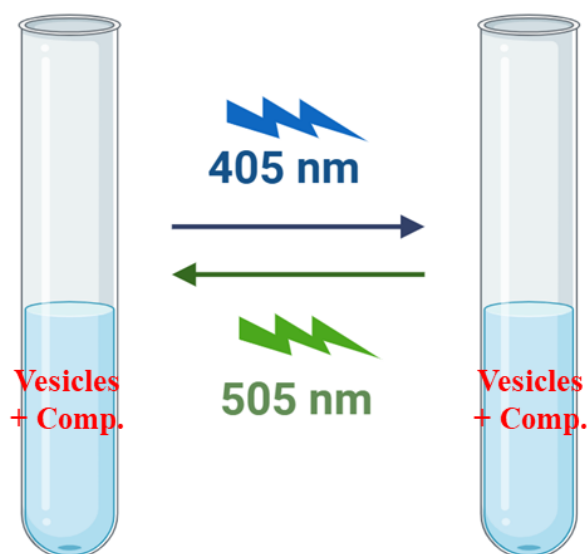


Figure S28: Photo-irradiation during the water transport assay showing the irradiation procedure with vesicle and compound 1.

6. Stopped Flow Experiment:^{S3} The water permeability of the channels was assessed using a stopped-flow instrument (SFM3000 + MOS450, Bio-Logic SAS, Claix, France). Upon exposure to a hypertonic osmolyte solution (400 mM sucrose) prepared in the same buffer, liposomes experienced shrinkage due to the establishment of an outward osmotic gradient. The resulting changes in liposome size were tracked by measuring light scattering intensity at 365 nm with a 90° detection angle, in accordance with the Rayleigh-Gans theory applied to the system. The experimental data were fitted using a sum of two exponential functions, and the osmotic permeability (P_f) was determined using the following equation:

$$P_f = k / (S/V_0) \cdot V_w \cdot \Delta_{\text{osm}} \quad \text{Equation S3}$$

where k is the exponential coefficient of the change in the light scattering; S and V_0 are the initial surface area and volume of the vesicles, respectively; V_w is the molar volume of water and Δ_{osm} is the osmolarity difference.

In this experiment, 100 μL of vesicles, prepared as described above, were suspended in 1880 μL of 200 mM sucrose solution in 10 mM PBS buffer (pH 6.4). A 20 μL aliquot of the test compound 1 (1 mM and 2 mM in DMSO) was then added to the suspension. Each sample were irradiated with 405 nm LED (for E) and 505 nm (for Z) and were maintained at 20°C for 30 minutes before being exposed to a 400 mM sucrose solution in the stopped-flow instrument (figure S28). In each case, the average water permeability was determined using Equation S3, based on permeability values obtained from at least three independent measurements.

7. Membrane Insertion Experiment:

Standard calibration curves for the 1-E and 1-Z isomers were generated following irradiation of the compound solutions (prepared in 200 mM sucrose and 10 mM PBS, pH 6.4) with 405 nm and 505 nm LEDs, respectively (Figures S29–S30). To evaluate membrane insertion efficiency, 20 μ L of a 2 mM stock solution of compound 1 was added to separate cuvettes containing 200 mM sucrose and PBS buffer. One cuvette was irradiated at 405 nm to generate the 1-E isomer, while the other was irradiated at 505 nm to generate the 1-Z isomer, prior to the addition of 100 μ L of PC/PS/cholesterol vesicle suspension. Following a 30-minute incubation, the mixtures were transferred into Float-A-Lyzer dialysis tubes (molecular weight cutoff: 3.5–5 kDa) containing 6 mL of 200 mM sucrose and PBS buffer. Unincorporated channels were allowed to diffuse out over a period of 3 hours, after which the vesicle suspensions were subjected to centrifugation for 30 minutes. The resulting supernatants were analyzed by UV–Vis spectroscopy. Based on the pre-established standard curves, the concentration of unincorporated channels was quantified, and membrane incorporation efficiency was calculated as the ratio of incorporated channel moles to the total amount initially added.

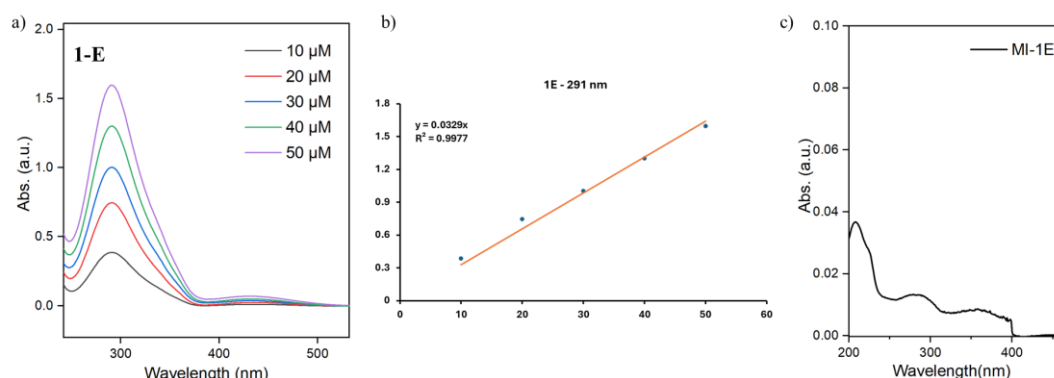


Figure S29: Standard concentration UV-Vis curves for 1-E (a), linear plot of the absorbance value of 1-E at 291 nm (b) and the UV-Vis absorbance spectrum recorded of the supernatant containing unincorporated E isomer (c).

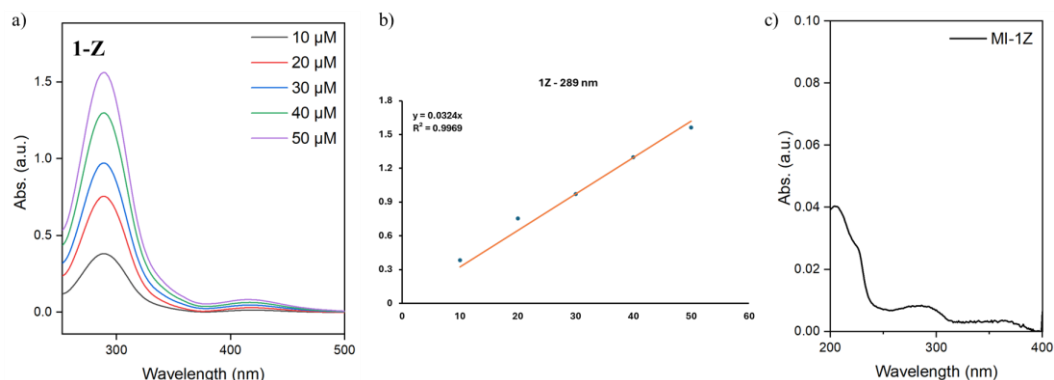


Figure S30: Standard concentration UV-Vis curves for 1-Z (a), linear plot of the absorbance value of 1-Z at 289 nm (b) and the UV-Vis absorbance spectrum recorded of the supernatant containing unincorporated Z isomer (c).

References:

- S1 A. Kerckhoffs and M. J. Langton, *Chem. Sci.*, 2020, **11**, 6325–6331.
- S2 T. G. Johnson, A. Sadeghi-Kelishadi and M. J. Langton, *J. Am. Chem. Soc.*, 2022, **144**, 10455–10461.
- S3 D. Su, A. Van Der Lee and M. Barboiu, *Angew Chem Int Ed*, 2025, **64**, e202413816.