Electronic Supplementary Information (ESI)

Reversible Photo-Gated Transmembrane Channel Assembled from Acylhydrazone-Contained Crown Ether Triad

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Contents

S1 General methods and materials

All chemicals were commercially available unless noted otherwise. Compounds **2**[1], **3**[2], **4**[1] were prepared according to the literatures procedure.

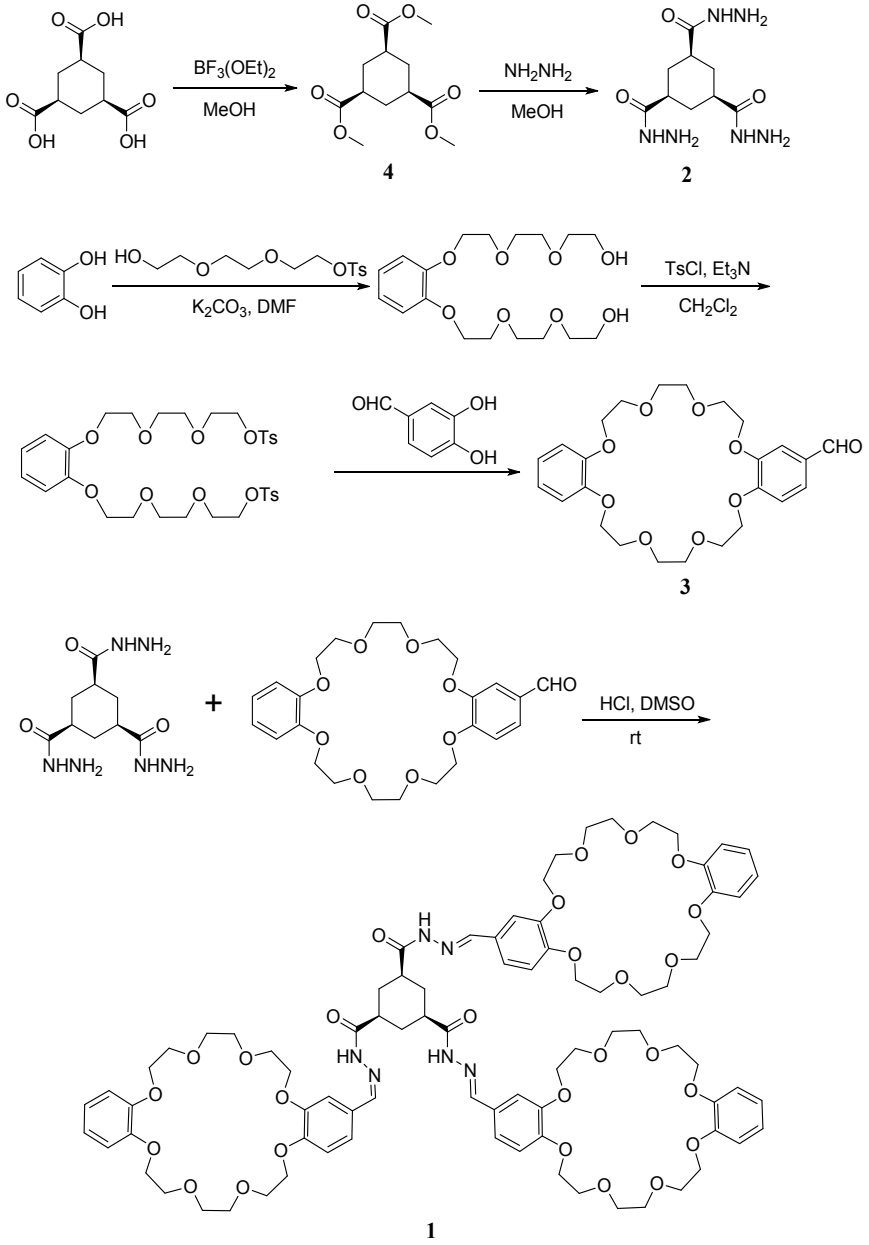

Figure S1. Synthetic routes of **1**.

S2 Synthesis and characterization of compounds

Preparation of 4.[1]

cis, cis-Cyclohexane-1,3,5-tricarboxylic acid (1.0 g, 4.6 mmol) was heated under reflux for 6 h with boron trifluoride diethyl etherate (4.2 g, 30 mmol) in an excess amount of dry methanol (50 mL). The reaction mixture was cooled and poured into a saturated sodium bicarbonate solution. The organic phase was then extracted with diethyl ether and dried over Na_2SO_4. Removal of ether afforded the crude trimethyl ester (1.0 g, 83% yield). ^{1}H NMR (400 MHz, d_6-DMSO) δ 2.87 (s, 9H), 1.69 (t, J = 11.4 Hz, 3H), 1.41 (d, J = 12.3 Hz, 3H), 0.61 (q, J = 12.5 Hz, 3H).

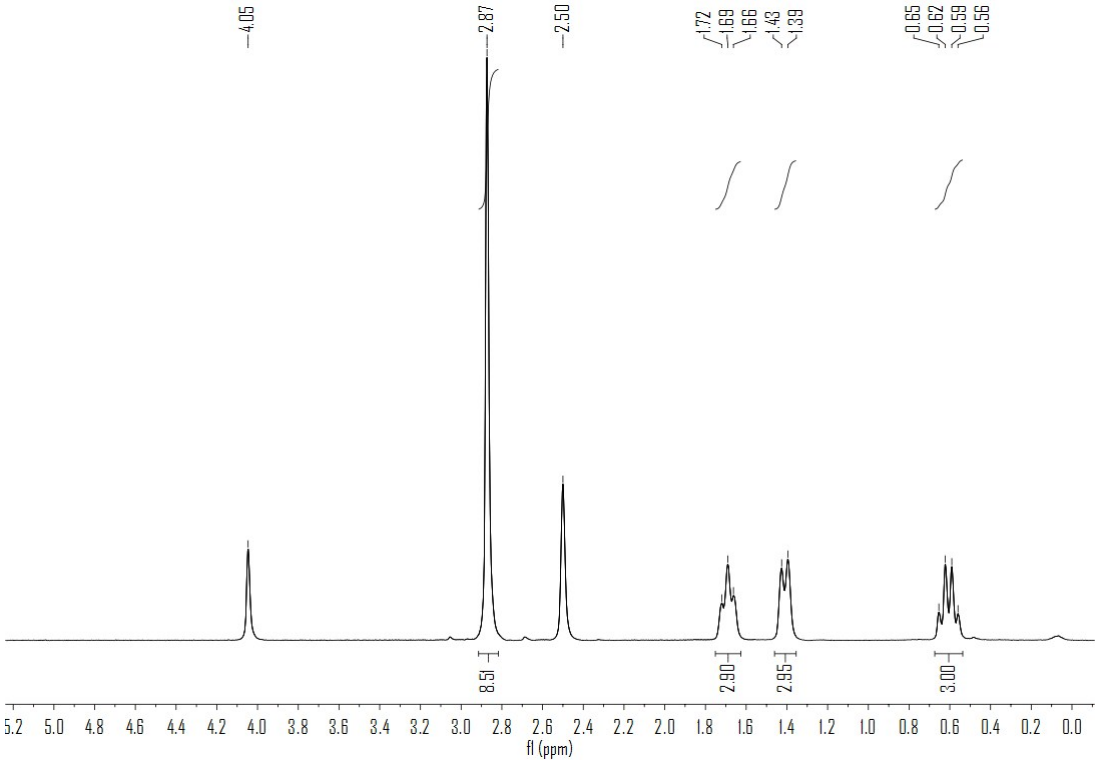

Figure S2. ^{1}H NMR spectrum of **4** (400 MHz, d_6-DMSO, 298 K).

Preparation of 2.

4 (1.0 g, 3.87 mmol) and hydrazine hydrate (3.51 mL, 64% in water) in methanol (15 mL) was stirred at room temperature for 3 h. When the reaction is complete, the solvents and hydrazine excess were evaporated in vacuo. the product was dried under vacuum to give **2** (1g,

3.87mmol, quant.) as a white powder. ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.95 (s, 3H), 4.15 (s, 6H), 2.11 (t, *J* = 11.8 Hz, 3H), 1.57 (d, *J* = 12.7 Hz, 3H), 1.47 (q, *J* = 12.3 Hz, 3H). ^{13}C NMR (100 MHz, d_6-DMSO) δ 174.0, 41.2, 31.8.

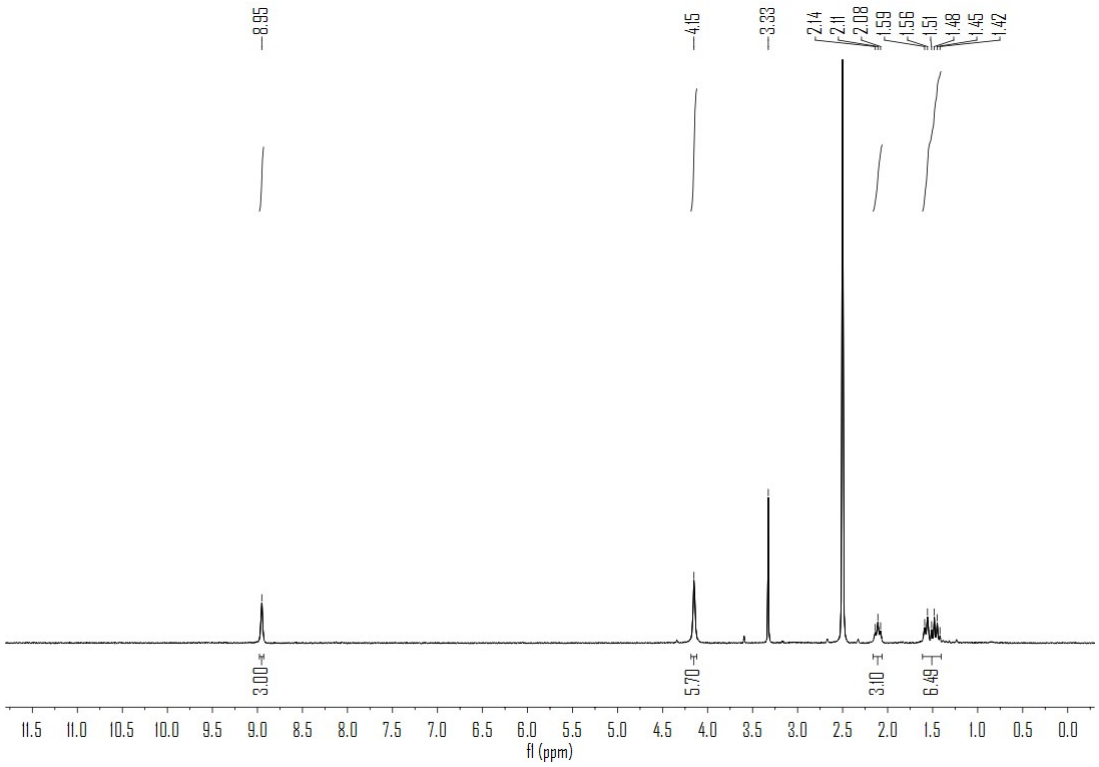

Figure S3. ^{1}H NMR spectrum of **2** (400 MHz, d_6-DMSO, 298 K).

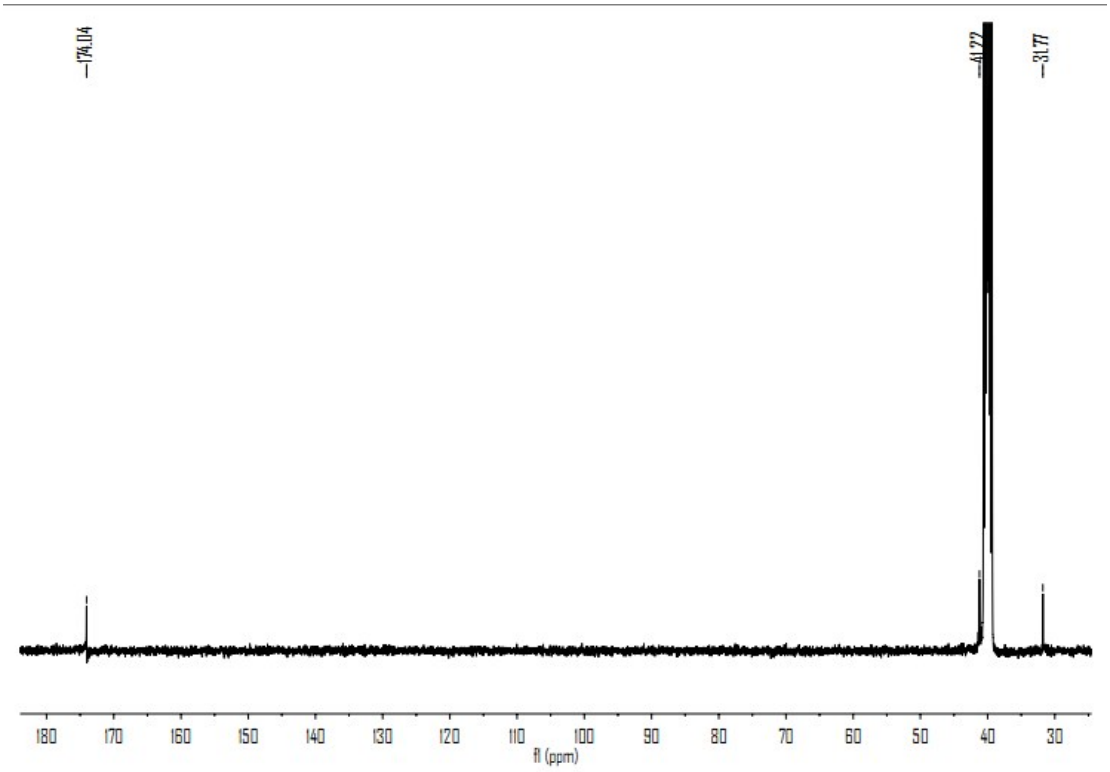

Figure S4.^{13}C NMR spectrum of **2** (100 MHz, d_6-DMSO, 298 K).

Preparation of 3.

3 was prepared according to the literatures procedure[2]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (s, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.37 (s, 1H), 6.93 (d, J = 8.2 Hz, 1H), 6.88 (m, 4H), 4.23-3.84 (m, 24H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.9, 154.3, 149.2, 148.9, 130.2, 126.8, 121.4, 121.4, 121.3, 113.9, 111.8, 110.9, 71.6, 71.5, 71.3, 69.9, 69.7, 69.5, 69.5, 69.3. HRMS (MALDI) m/z ($M+Na^+$) calcd for $C_{25}H_{32}O_9Na^+$: 499.1944. Found: 499.1942.

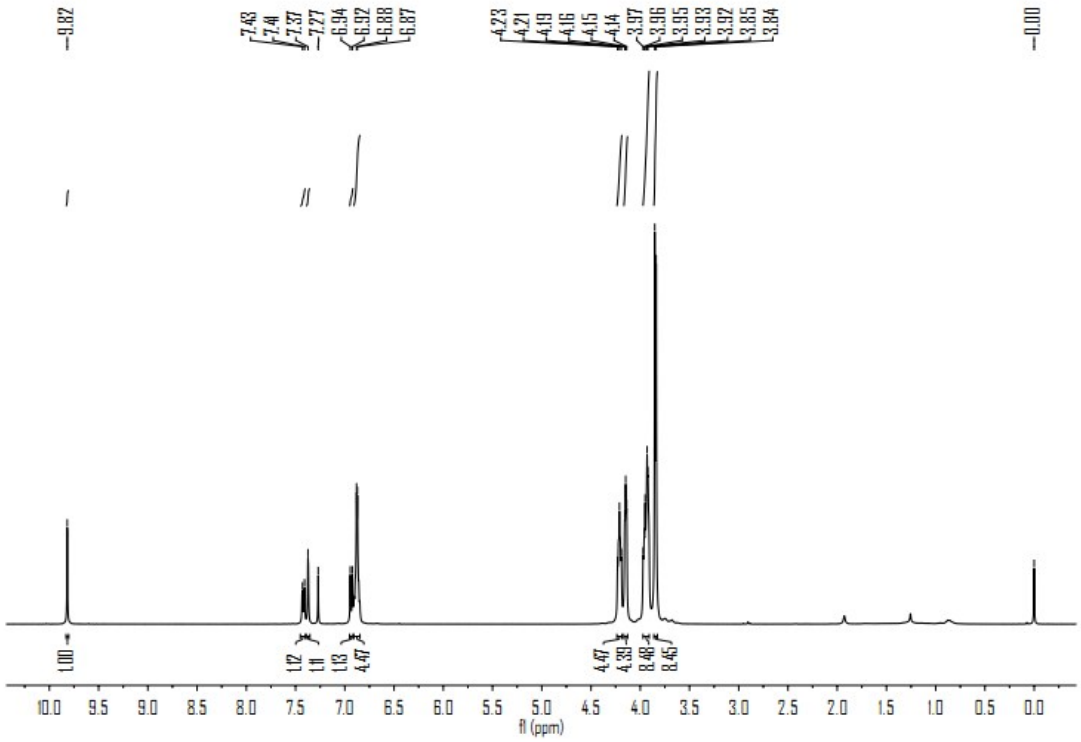

Figure S5. ^{1}H NMR spectrum of **3** (400 MHz, $CDCl_3$, 298 K).

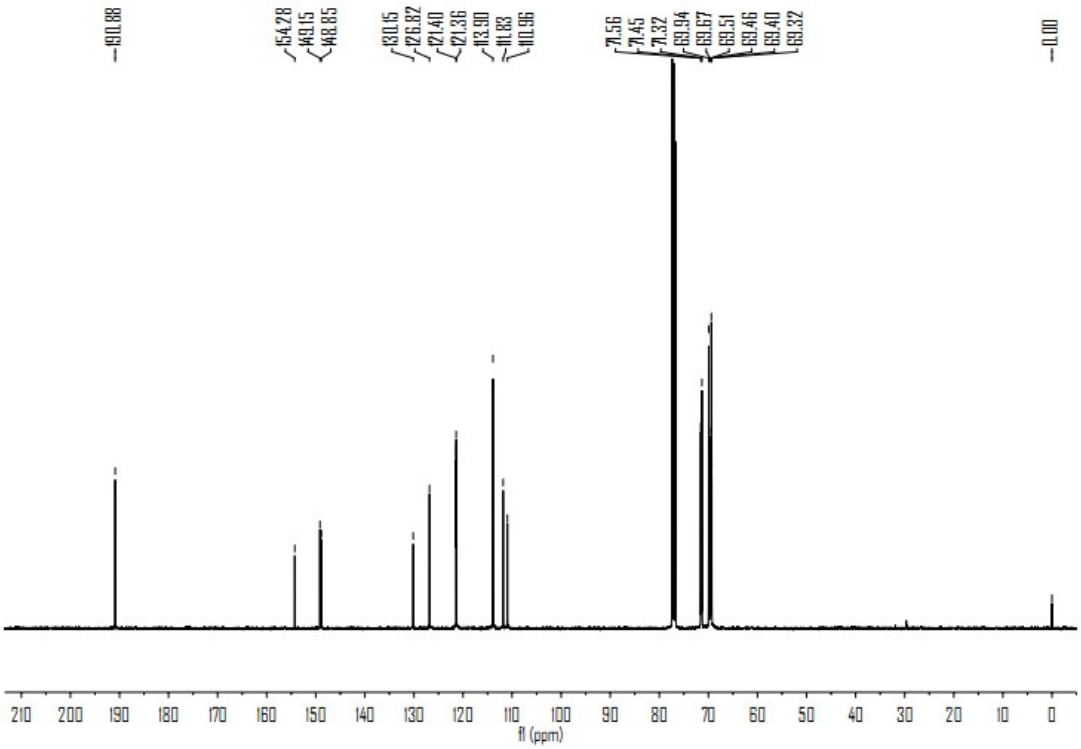

Figure S6. ^{13}C NMR spectrum of **3** (100 MHz, $CDCl_3$, 298 K).

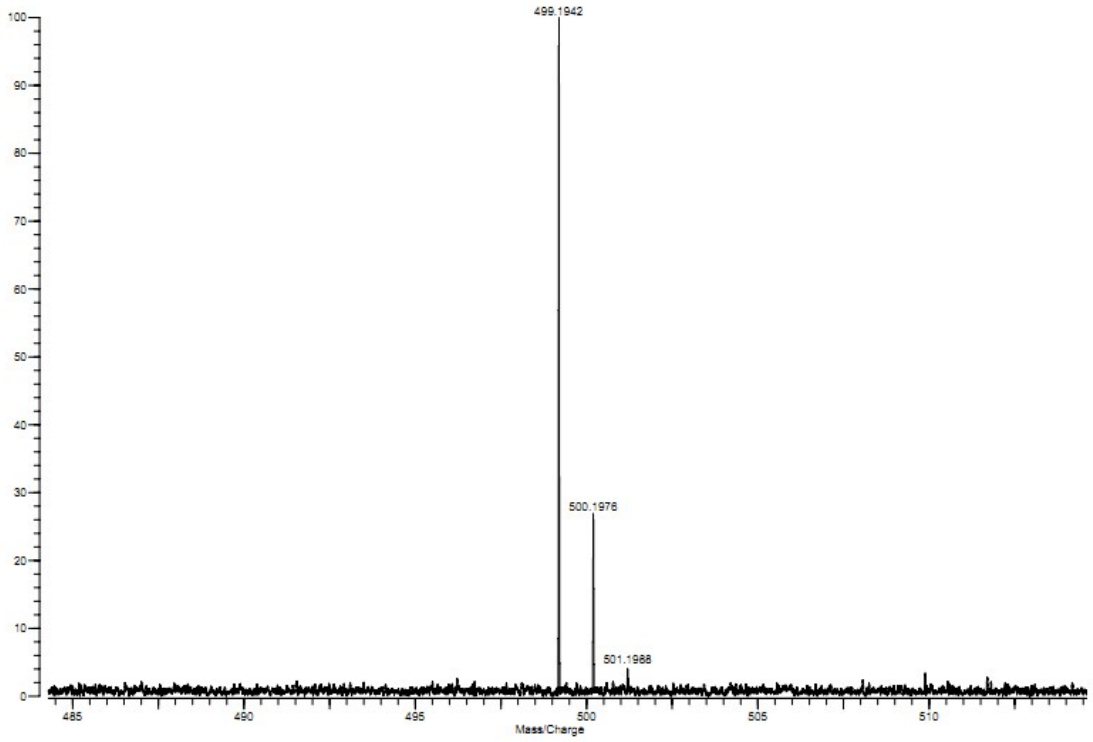

Figure S7. MALDI-TOF mass spectrum of compound **3**.

Preparation of 1.

To a solution of **2** (30.0 mg, 0.12 mmol) and **3** (221.4 mg, 0.48 mmol) in DMSO (10 mL) was added a drop of HCl (20%), the solution was stirred at room temperature for 24 h. When the reaction is complete, the reaction mixture was poured into water (500 mL). The aqueous layer was then extracted with CH_2Cl_2 (2×500 mL), and the combined organic phase was dried over Na_2SO_4. After removal of solvent, the crude product was recrystallized twice from methanol to provided **1** (182.2mg, 0.11mmol, 96%) as a white powder. ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.25 (s, 1.53 H), 11.17 (s, 1.35 H), 8.09 (s, 1.52 H), 7.89 (s, 1.37H), 7.26-7.15 (m, 6H), 6.99-6.87 (m, 15H), 4.10-3.60 (m, 72H), 1.92 (br, 3H), 1.44 (br, 6H). ^{13}C NMR (100 MHz, d_6-DMSO) δ 175.91, 170.76, 150.67, 150.35, 148.93, 147.08, 127.77, 122.24, 122.18, 121.61, 114.52, 113.57, 112.27, 112.23, 110.82, 70.95, 70.88, 69.63, 69.48, 69.18, 42.11, 30.71. δ.

FT-IR (KBr, cm^{-1}): 3204.01, 3061.87, 2923.94, 2871.43, 1663.37, 1597.93, 1506.97, 1452.23, 1264.16, 1132, 1052.54, 938.65, 858.80, 815.51, 746.75. HRMS (MALDI) m/z ($M+Na^+$) calcd for $C_{84}H_{108}N_6O_{27}Na^+$: 1655.7160. Found: 1655.7158.

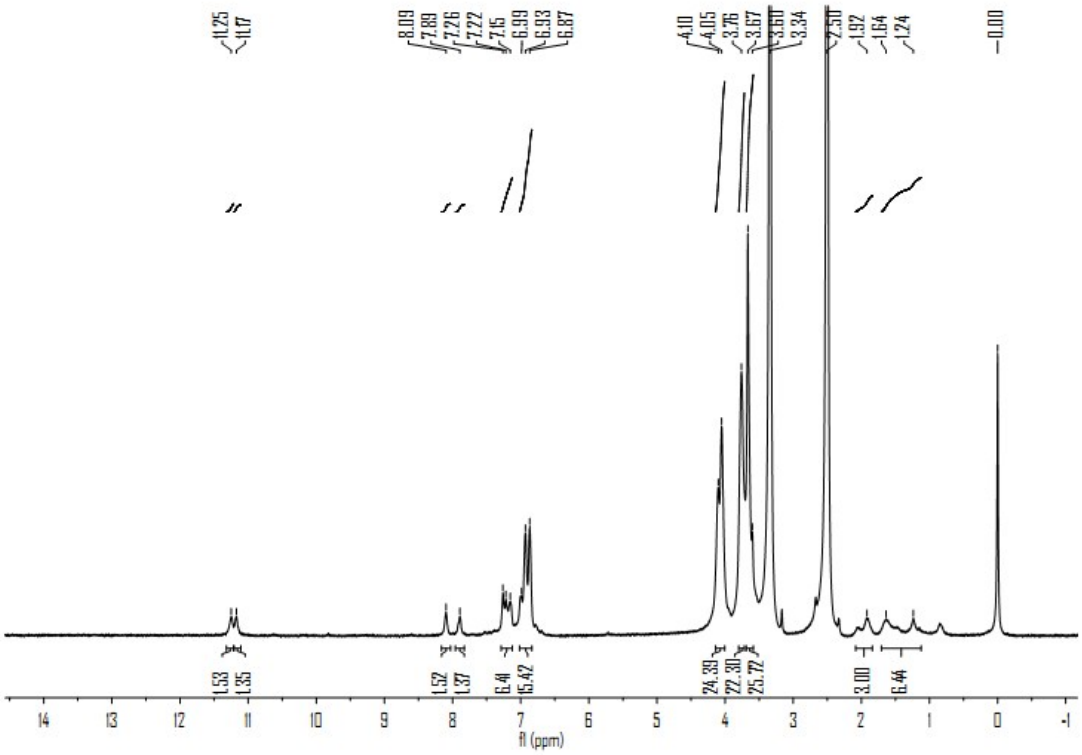

Figure S8. ^1H NMR spectrum of **1** (400 MHz, d_6-DMSO, 298 K).

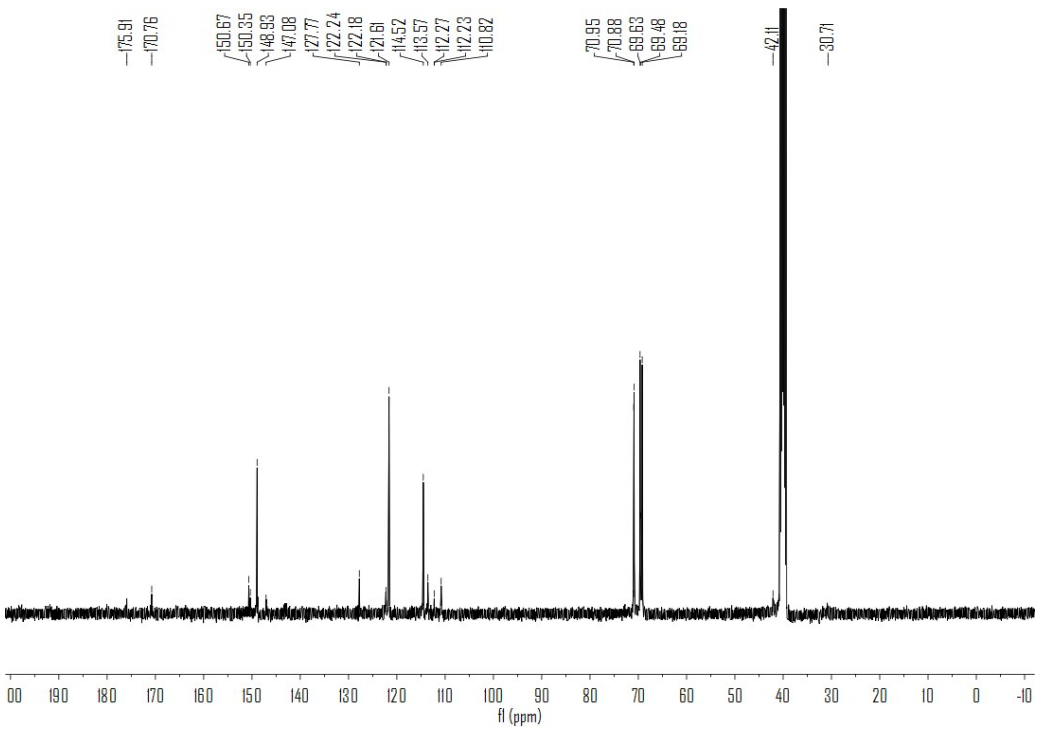

Figure S9. ^{13}C NMR spectrum of 1 (100 MHz, d_6-DMSO, 298 K).

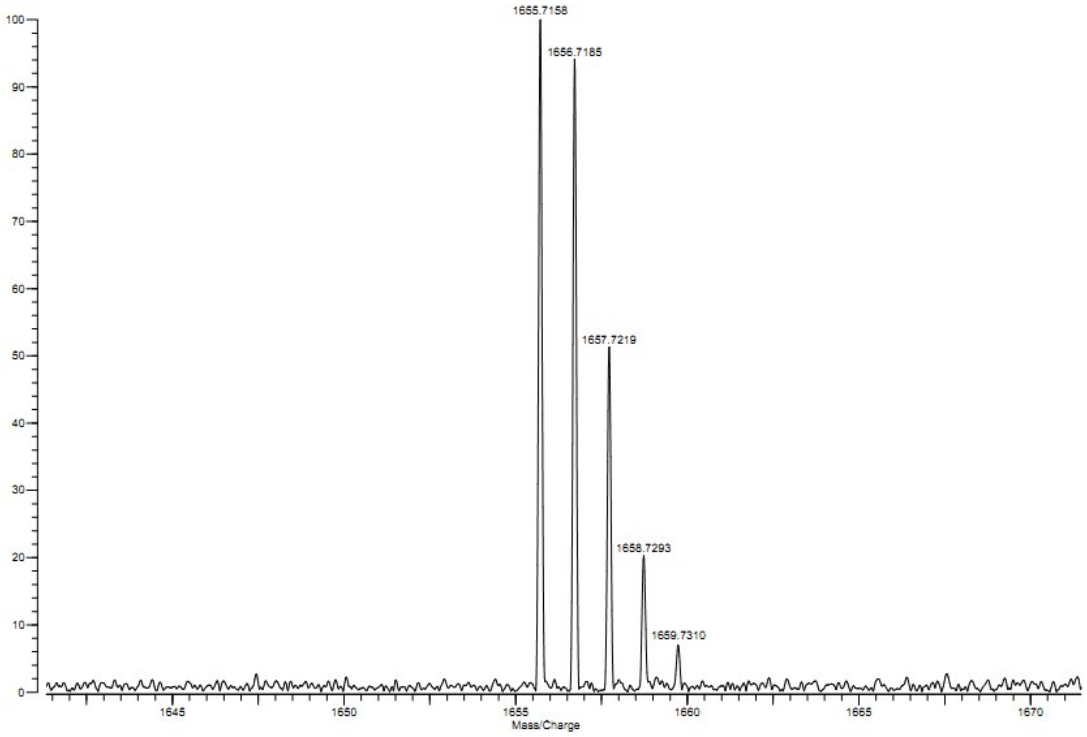

Figure S10. MALDI-TOF mass spectrum of the compound of **1**.

S3 Photochromic Properties of compound 1.

It is well-documented that the acylhydrazone scaffold is photo-responsive, and its (*E*)-isomers can transform to (*Z*)-isomers under UV light irradiation. In addition, the *E*/*Z* isomerization process is reversible, and the reverse process can be actuated under visible light irradiation or extended annealing under dark conditions (Figure S11). As shown in Figure S12a, the UV-vis spectrum of (*E*)-isomer of **1** displayed an absorption band at λ_{max} = 317 nm. After UV light irradiation (λ_{irr} = 320 nm, t_{irr} = 5 min), this absorption band showed the obvious decrease of intensity, accompanied by the hypochromic shifts of absorption maximums, while a new weak absorption band at λ_{max} = 360 nm developed (Figure S12b), indicating the isomerization of (E)-isomer to (Z)-isomer. Interestingly, the decreased and hypochromically shifted absorption peaks could recover the original level when further irradiation (λ_{irr} = 365 nm, t_{irr} = 5 min) the UV-irradiated solution, owing to the reversible transformation (*Z*)-isomer to (*E*)-isomer. (Figure S12c). Interestingly, this reversible photo/thermal-switched isomerization cycles could be repeated for tens of times without any fatigue (Figure S12d).

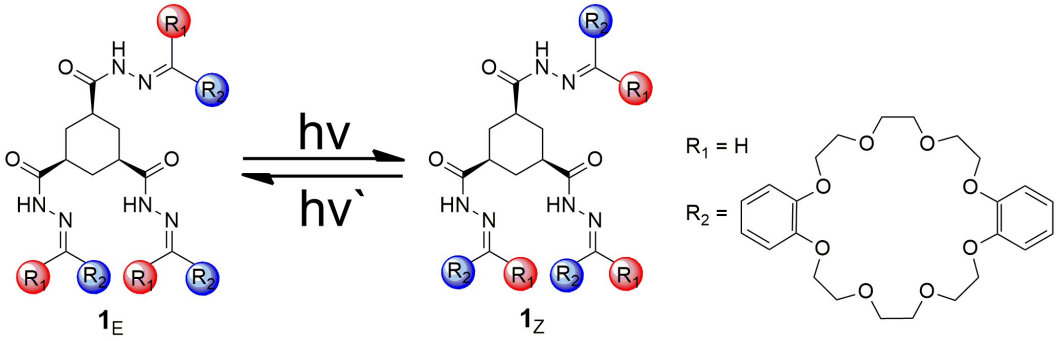

Figure S11. General structure and isomerization of acylhydrazone photoswitches.

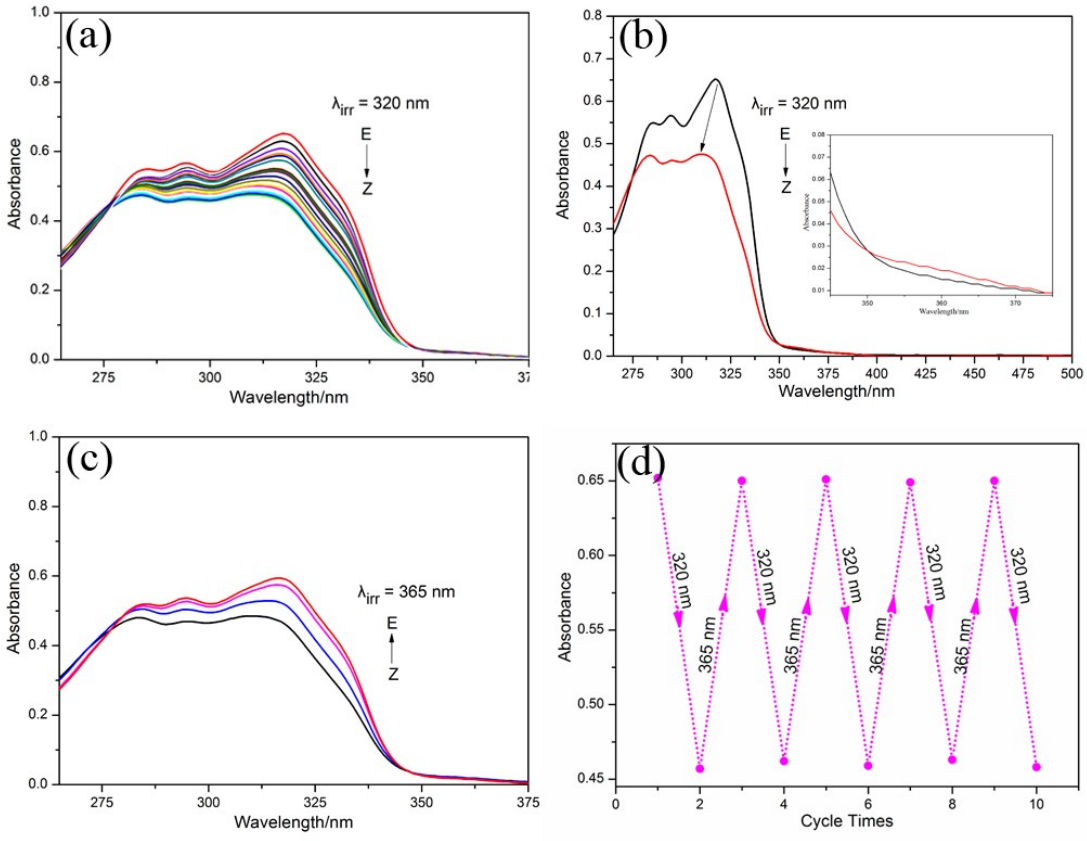

Figure S12. (a) Absorption spectra variation of **1** upon irradiation by UV light (320 nm). (b) Absorption spectra variation of **1** upon irradiation by UV light (320 nm) and (inset) expansion of λ = 330-380 nm. (c) UV/Vis spectra of conversion of $\mathbf{1}_{Z}$ to $\mathbf{1}_{E}$ under light irradiation at 365nm. (d) Absorption changes of **1** at 317 nm under UV light irradiation 320 nm and 365 nm. [**1**] = 0.01 mM (in $CHCl_3$ solution).

S4 Procedures for the patch clamp experiments.

The solution of diphytanoylphosphatidylcholine (diPhyPC) in chloroform (10 mg/ml, 20 μL) was evaporated with nitrogen gas to form a thin film and re-dissolved in n-decane (5 μL). The lipid solution (0.5 μL) was injected onto the aperture (diameter = 200 μm) of the Delrin® cup (Warner Instruments, Hamden, CT) and then evaporated with nitrogen gas. In a typical experiment for the measurement of channel conductance for an ion, the chamber (*cis* side) and

the Delrin cup (*trans* side) were filled with aqueous KCl solution (1.0 M, 1.0 mL). Ag-AgCl electrodes were applied directly to the two solutions and the *cis* one was grounded. Planar lipid bilayer was formed by painting the lipids solution (1.0 μL) around the pretreated aperture and by judgment of capacitance (80-120 pF). The solution of the test compounds **1** in DMSO (1 μM, 1 μL) was added to the *cis* chamber and the solution was stirred for 5 min. Membrane currents were measured using a Warner BC-535D bilayer clamp amplifier and were collected by PatchMaster (HEKA) with sample interval at 5 kHz and then filtered with a 8-pole Bessel filter at 1 kHz (HEKA). The data were analyzed by FitMaster (HEKA) with a digital filter at 100 Hz.

For the measurement of the transport selectivity of K^+ over Cl^-, the *cis* and *trans* chambers were charged with KCl of 0.3 M and 1.0 M, respectively. The reversal potential (ε_{rev}) of **1** under this condition was determined from the *I-V* plot (Figure S13). By using this ε_{rev} value, its transport selectivity of K^+ over Cl^-, defined as the permeability ratio P_K / P_{Cl}, was calculated from the Goldman−Hodgkin−Katz equation (Figure S13, insect).

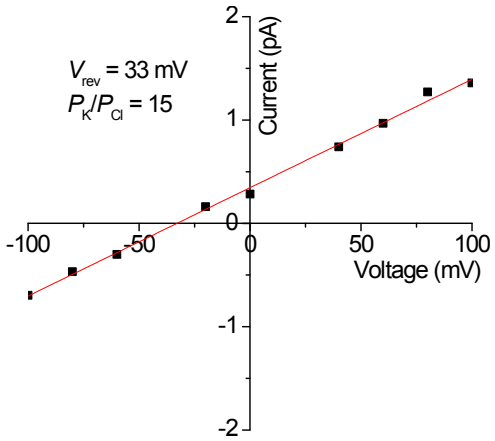

Figure S13. *I-V* plot of **1** in the lipid bilayer (*cis* chamber: KCl (0.3 M); *trans* chamber: KCl (1.0 M)). Insect: the calculated ε_{rev} and P_K / P_{Cl} of **1**.

For the measurement of the transport selectivity of M^+ (M = Rb, NH_4, Cs, Na) over K^+, the *cis* and *trans* chambers were charged with KCl (1.0 M) and MCl (1.0 M), respectively. The reversal potentials (ε_{rev}) of **1** under this condition were determined from the *I-V* plots (Figure S14). By using these ε_{rev} values, its transport selectivity of M^+ over K^+, defined as the permeability ratio P_M / P_K, was calculated from the Goldman−Hodgkin−Katz equation (Figure S14, insect).

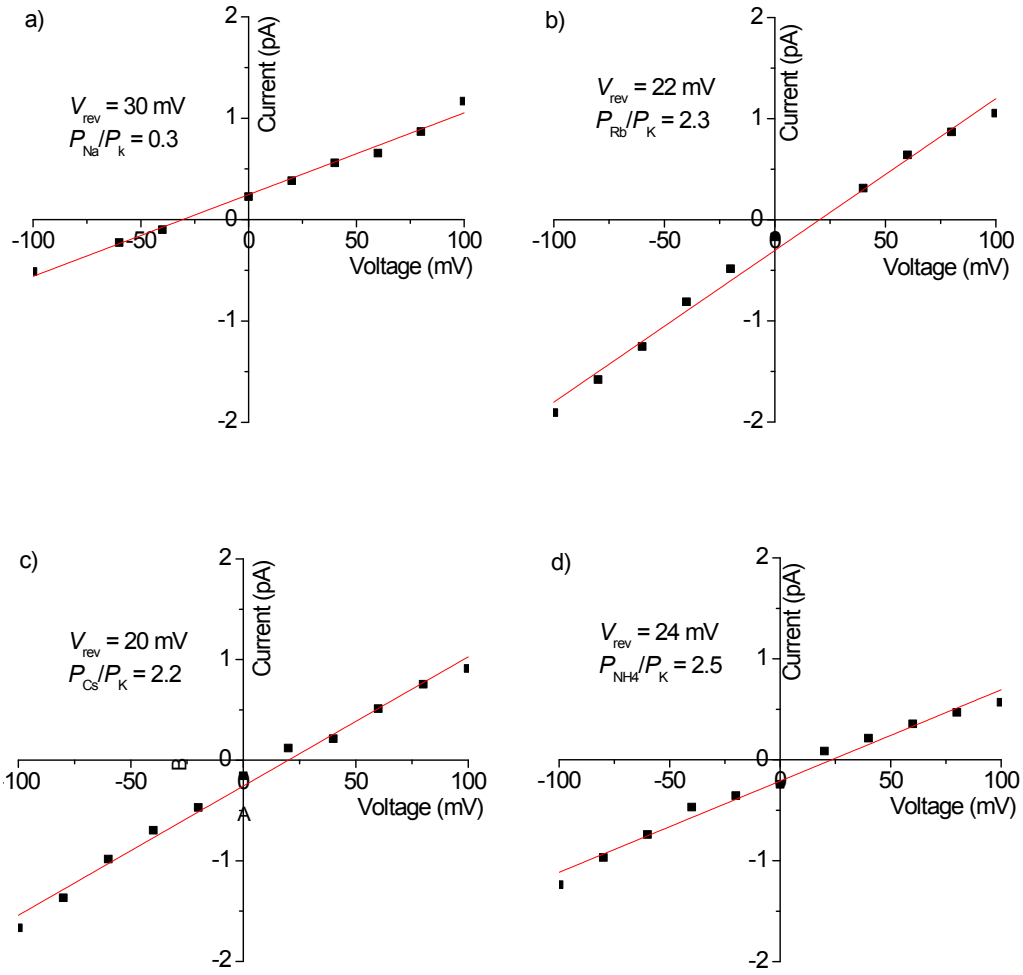

Figure S14. *I-V* plot of **1** in the lipid bilayer (*cis* chamber: KCl (1.0 M); *trans* chamber: MCl (1.0 M)). (a) M = Na^+; (b) M = Rb^+; (c) M = Cs^+; (d) M = NH_4^+. Insect: the calculated ε_{rev} and

P_K / P_{Cl} of **1**.

References:

1. Boekhoven, J.; Poolman, J. M.; Maity, C.; Li, F.; Mee, L. Minkenberg, C. B.; Mendes, E.; Esch, J. H.; Eelkema, R. *Nat. Chem.* 2013, **5**, 433.
2. Cantrill, S. J.; Youn, G. J.; Stoddart, J. F.; Williams, D. J. *J. Org. Chem.* 2001, **66**, 6857–6872.
3. Poolman, J. M.; Boekhoven, J.; Besselink, A.; Olive, A. G. L.; Esch, J. H.; Eelkema, R. *Nat. Protoc.* 2014, **9**, 977.