Toward transmembrane anionophores based on rigid bis(choloyl) conjugates: reversal of the ion selectivity by appended polyamines

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Supporting Information

Experimental

Generals. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker Avance AV 400 spectrometer and the solvents as standards. ESI and HR mass spectra were measured on Waters UPLC/Quattro Premier XE and Bruker maXis 4G ESI-Q-TOF mass spectrometers, respectively. IR spectra were recorded on a Brucker TENSOR 27 FTIR instrument using KBr discs in the range of 400-4000 cm^{-1}. Silica gel 60 Å (reagent pure, Qingdao Haiyang Chemical Co. Ltd) was used for column chromatography. Analytical thin-layer chromatography (TLC) was performed on silica gel plates 60 GF254. Detection on TLC was made by use of iodine, UV (254 or 365 nm) and 20% aqueous H_2SO_4. Fluorescence spectra were measured on a Shimadzu RF5301PC spectrofluorimeter. Chloride efflux was measured on a Mettler-Toledo PerfectIon^{TM} chloride ion selective electrode.

EYPC and pyranine were purchased from Sigma Chemical Co. (St Louis, USA). Tri-Boc protected spermine was prepared according to reported protocols. [1] All the other chemicals and reagents were obtained from commercial sources and used without further purification. Buffer solutions were prepared in triply distilled deionized water.

Synthesis of compound **1**

*Synthesis of Boc-protected compound **1***. To a solution of compound **2** (100 mg, 0.09 mmol) and NHS (68 mg, 0.60 mmol) in DMF (2 mL) was added DCC (123 mg, 0.60 mmol). The resulting mixture was stirred at room temperature and checked with TLC ($CHCl_3$/CH_3OH/ H_2O, 180/20/1, v/v/v). After 4 h, a solution of tri-Boc protected spermine (171 mg, 0.34 mmol) in DMF (1 mL) was added. The resulting mixture was stirred at room temperature overnight and concentrated under reduced pressure. The obtained residues were purified by chromatography on a silica gel column, eluted with $CHCl_3$/EtOH/H_2O (300/40/1, v/v/v) to give Boc-protected compound **1** (98 mg, 54%) having main IR bands (KBr, cm^{-1}) υ 2929.9 (s), 2865.1 (s), 1672.9 (s), 1524.4 (s), 1476.5 (w), 1419.6 (s), 1249.8 (s), 1166.5 (s); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.26 (s, 4H), 4.43-4.34 (m, 6H), 3.97 (s, 2H), 3.82 (s, 2H), 3.40 (m, 2H), 3.24-3.17 (m, 20H), 3.05 (t, J = 6.0 Hz, 4H), 2.33-1.04 (m, 132H), 0.94 (s, 6H), 0.72 (s, 6H); ESI-MS: *m/z* 2168.2 ($[M+Na]^+$), 1095.9 ($[M+2Na]^{2+}$) and HR-ESI-MS for $C_{116}H_{199}N_{12}O_{24}$ ($[M+H]^+$), calcd: 2144.4715; found: 2144.4739.

*Synthesis of compound **1***. To a solution of Boc-protected compound **1** (45 mg, 0.02 mmol) in CH_2Cl_2 (2 mL) was added TFA (0.89 mL). The mixture was stirred at room temperature for 3.5 h, and concentrated under reduced pressure. The obtained residues were dissolved in CH_3OH (0.3 mL) and transferred to a centrifuging tube. Ammonia solution (5%, 60 mL) was added and the resulting mixture was centrifuged for 20 min. The clear upper solution was disposed and water (10 mL) was added. The resulting mixture was re-centrifuged for 5 min. The obtained precipitates were dissolved in CH_3OH and concentrated under reduced pressure to afford compound **1** (10 mg, 32%) having main IR bands (KBr, cm^{-1}) υ 3282.4 (br), 3065.3 (m), 2928.8 (s), 2863.0 (m), 2357.6 (s), 1652.1 (s), 1540.3 (s), 1456.8 (w), 1371.8 (m), 1307.8 (m), 1200.0 (m); ^{1}H-NMR (CD_3OD, 400 MHz) δ 7.26 (s, 4H), 4.38-4.35 (m, 6H), 3.97 (s, 2H), 3.82 (s, 2H), 3.40 (m, 2H), 3.28-3.19 (m, 4H), 2.81-2.64 (m, 20H), 2.36-1.04 (m, 78H), 0.94

(s, 6H), 0.72 (s, 6H); ^{13}C-NMR (CD_3OD, 100 MHz) δ 175.5, 173.5, 172.4, 137.3, 127.2, 72.6, 71.4, 67.6, 52.9, 48.8, 48.7, 48.4, 46.7, 46.6, 46.1, 46.0, 42.3, 41.7, 41.6, 39.6, 39.1, 39.0, 36.6, 35.6, 35.0, 34.4, 32.5, 31.8, 30.1, 29.8, 28.5, 28.2, 27.6, 26.5, 26.4, 22.8, 21.7, 16.4, 11.6; ESI-MS: *m/z* 1544.8 ($[M+H]^+$), 773.6 ($[M+2H]^{2+}$) and HR-ESI-MS for $C_{86}H_{150}N_{12}O_{12}$ ($[M+H]^+$) Calcd: 1544.1569; Found: 1544.1565.

EYPC vesicle preparations

Vesicles for pH discharge were prepared according to the reported protocols. [2] Specifically, EYPC (20 mg) was dissolved in chloroform (0.5 mL) in a pyrex test tube. While rotating the tube, the organic solvent was removed under a stream of nitrogen, resulting in a thin lipid film. The last traces of solvent were then removed under reduced pressure (room temperature, > 4 h). To the dried lipid film was added 1.0 mL of a 0.1 mM pyranine solution in 25 mM HEPES buffer (50 mM NaCl, pH 7.0), and the mixture was vortexed for 1 min. The dispersion was then incubated at room temperature for 5 min, followed by another 1 min of vortexing and 20 min of incubation at ambient temperature. The sample was subjected to seven freeze/thaw cycles (77 K/325 K), followed by extrusion through a 100 nm Nuclepore membrane (15 times). After extrusion, the dispersion was incubated at room temperature for 1 h. The non-entrapped pyranine was removed *via* gel filtration (Sephadex G-25, eluted with 25 mM HEPES buffer (50 mM NaCl, pH 7.0)).

Vesicles for chloride efflux experiments were prepared in a similar way, except that they were formed in 25 mM HEPES buffer (500 mM NaCl, pH 7.0) and that 25 mM HEPES (500 mM $NaNO_3$ or 250 mM Na_2SO_4, pH 7.0) was used to elute the Sephadex G-25 column to remove non-entrapped NaCl.

Vesicles for ion selectivity experiments were prepared in a similar way, except that they were formed in a 0.1 mM pyranine solution in 25 mM HEPES buffer (pH 7.0) containing 50 mM

sodium salt (= $NaNO_3$, NaCl, NaBr or NaI), or chloride salt (= LiCl, NaCl, KCl, RbCl or CsCl), and that 25 mM HEPES buffer (pH 7.0) containing 50 mM of the corresponding salt was used to elute the Sephadex G-25 column to remove non-entrapped pyranine.

Experimental procedures for pH discharge experiments

The pH discharge experiments were conducted by using the methods similar to those described previously. [3] Specifically, to 1.68 mL of 25 mM HEPES buffer (50 mM NaCl, pH 8.0) was added the vesicle dispersion (0.3 mL), followed by the addition of compound **1** in DMSO (20 μL). The appearance of fluorescent intensity (FI) was monitored as a function of time (ex 460 nm, em 510 nm, ex/em 3.0 nm/3.0 nm). After 20 min, 5 wt% aqueous Triton X-100 (50 μL) was added. The ion-transporting ability was estimated from the relative FI that was calculated using the following equation: relative FI (%) = $(I–I_0)/(I_{total}–I_0)\times100$, where I_0, I and I_{total} represent the fluorescence intensities of the dispersion at the initial time, after a period of time and after addition of 5 wt% aqueous Triton X-100, respectively. The initial rate constants (k_{in}'s) were obtained by fitting the initial data points to a linear curve.

The ion selectivity experiments were measured in a similar way, except that 25 mM HEPES buffer (pH 8.0) containing 50 mM of the corresponding salt under study was used.

Experimental procedures for chloride efflux experiments

Chloride efflux experiments were conducted by using the methods similar to those described previously. [4] Specifically, to 1.18 mL of a 25 mM HEPES buffer (500 mM $NaNO_3$, pH 7.0) was added the vesicle dispersion (0.3 mL), followed by the addition of compound **1** of varying concentrations in DMSO (20 μL). The chloride efflux was monitored as a function of time by using chloride ion selective electrode. After 5 min, 5 wt% aqueous Triton X-100 (50 μL) was added. The relative chloride efflux was calculated using the following equation:

relative chloride efflux (%) = $([Cl^-]–[Cl^-]_0)/([Cl^-]_{total}–[Cl^-]_0)\times100$, where $[Cl^-]_0$, $[Cl^-]$ and $[Cl^-]_{total}$ represent the concentrations of chloride ion at the initial time, after a period of time and after addition of 5 wt% aqueous Triton X-100, respectively.

The chloride efflux inhibition experiments were conducted in a similar fashion, except that the vesicle dispersion was added to a 25 mM HEPES buffer (pH 7.0) containing Na_2SO_4 (250 mM).

References

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2. W.-H. Chen, S. L. Regen, *J. Am. Chem. Soc.* **2005**, *127*, 6538.
3. W.-H. Chen, J. Zhou, Y. M. Wang, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4010.
4. J. T. Davis, P. A. Gale, O. A. Okunola, P. Prados, J. C. Iglesias-Sanchez, T. Torroba, R. Quesada, *Nat. Chem.* **2009**, *1*, 138.

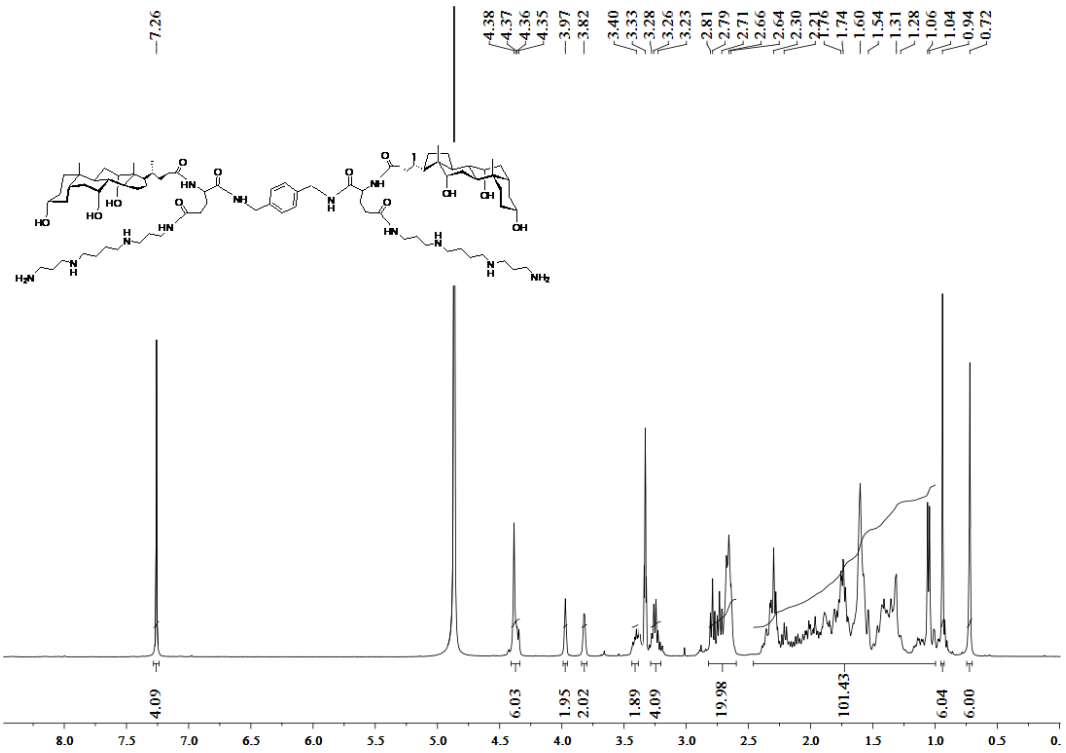


Fig. S1. ^{1}H-NMR (400 MHz, CD_3OD) of compound **1**.

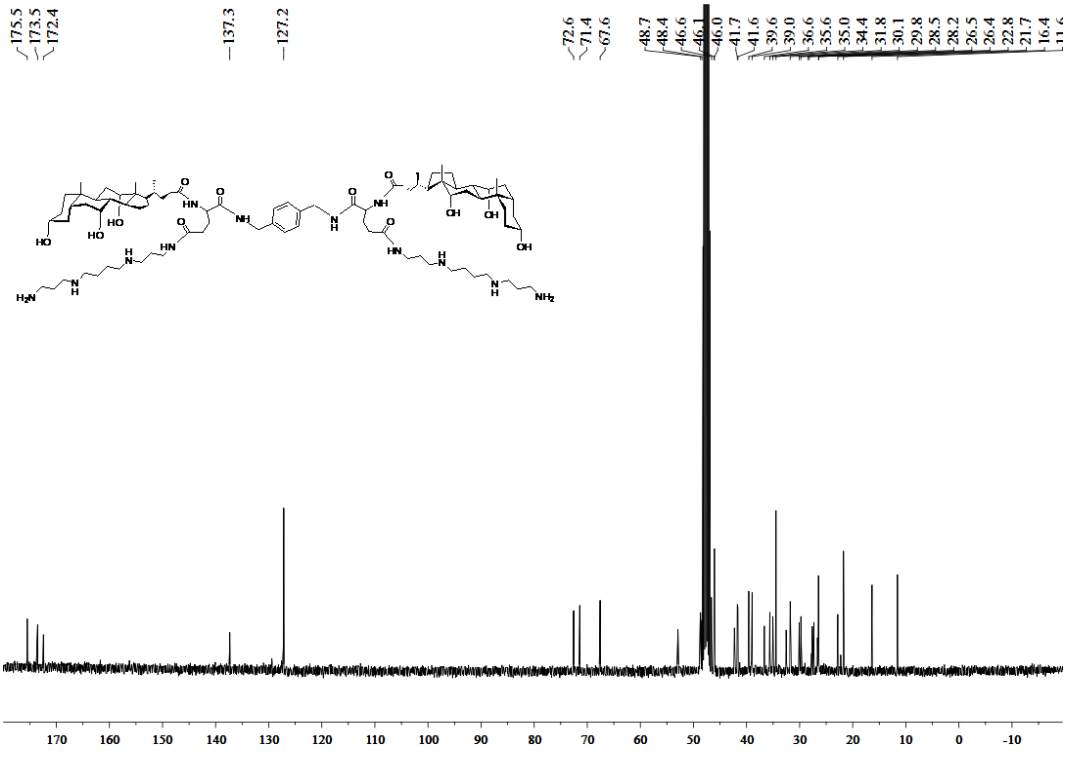


Fig. S2. ^{13}C-NMR (100 MHz, CD_3OD) of compound **1**.

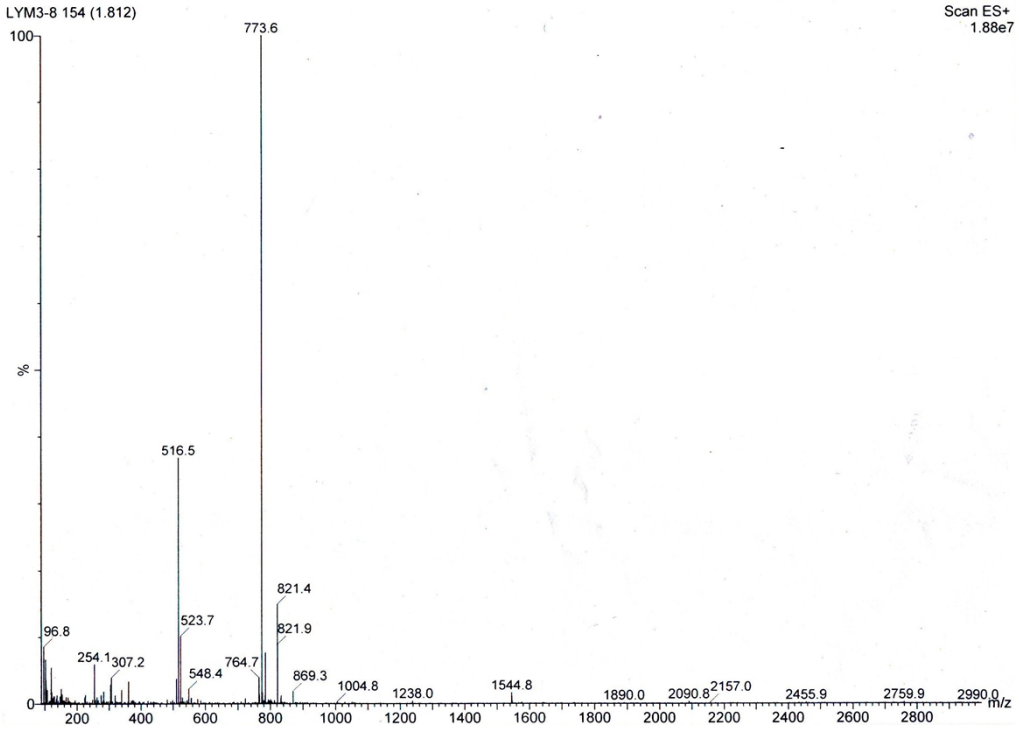
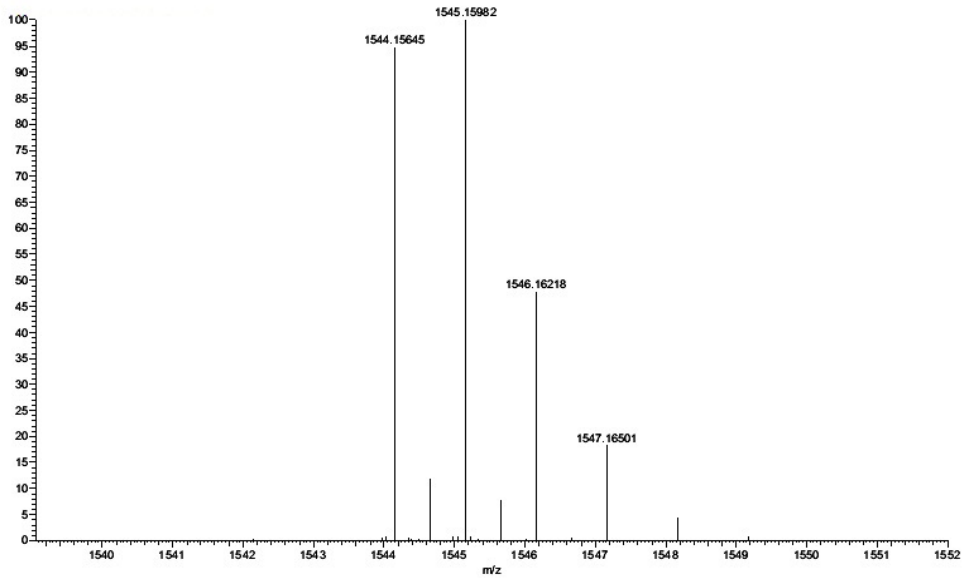


Fig. S3. ESI-MS of compound **1**.



SPECTRUM - simulation :

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1544.15645	1544.15689	-0.29	17.5	C86 H151 O12 N12

Fig. S4. HR-ESI-MS of compound **1**.

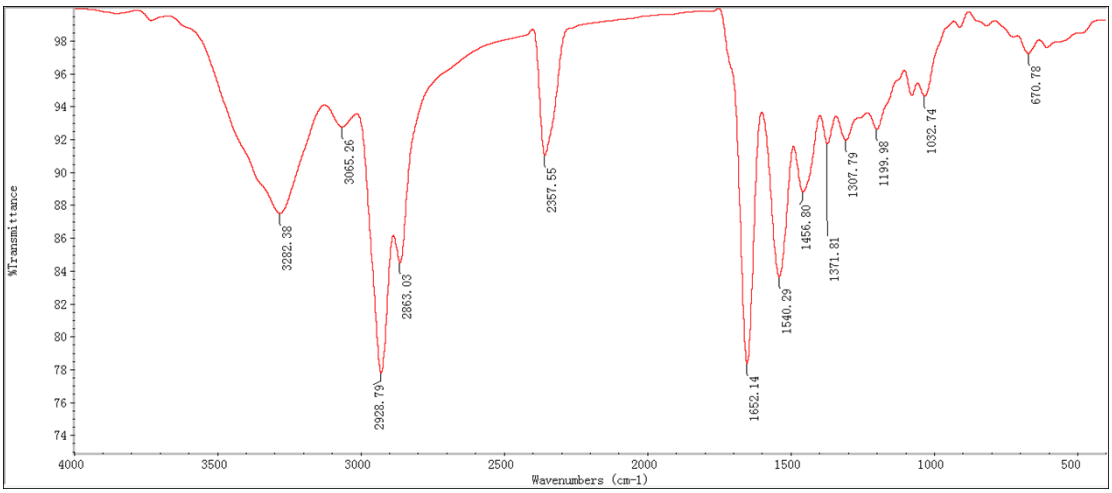


Fig. S5. IR of compound **1**.

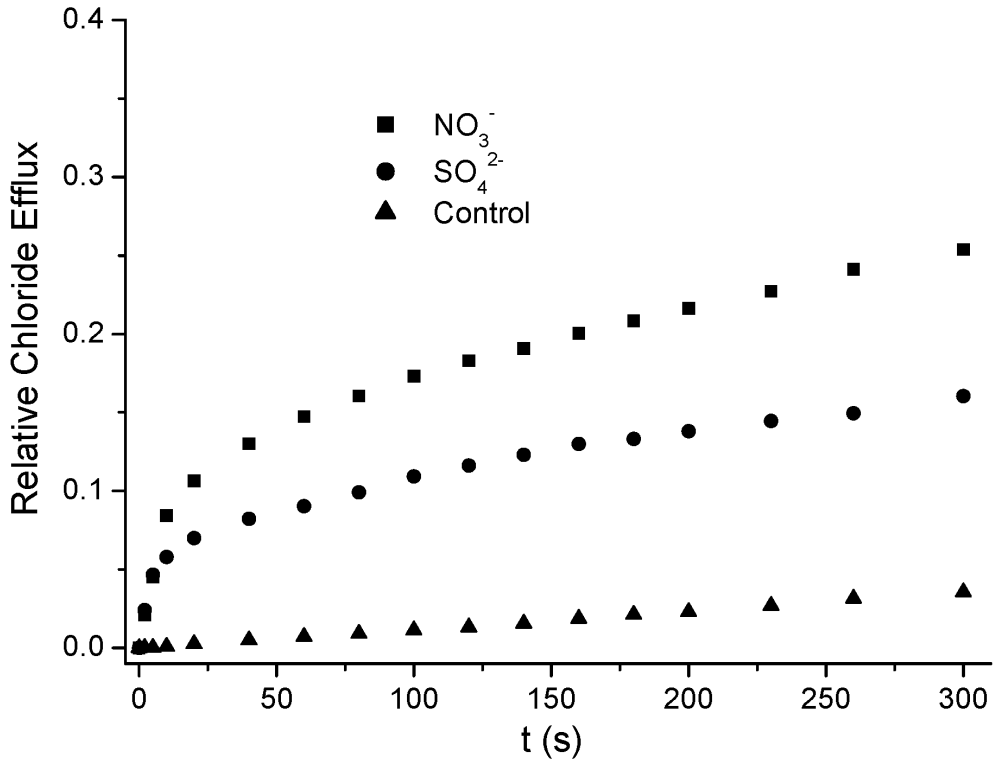


Fig. S6. Chloride efflux promoted by compound **1** of 5 mol% in EYPC vesicles (1.33 mM phospholipid) loaded with 500 mM NaCl buffered to pH 7.0 with 25 mM HEPES. The vesicles were dispersed in 25 mM HEPES buffer (pH 7.0) containing 500 mM $NaNO_3$ and 250 mM Na_2SO_4, respectively. The experiment that was conducted in Na_2SO_4 media and in the absence of compound **1**, was used as a control.

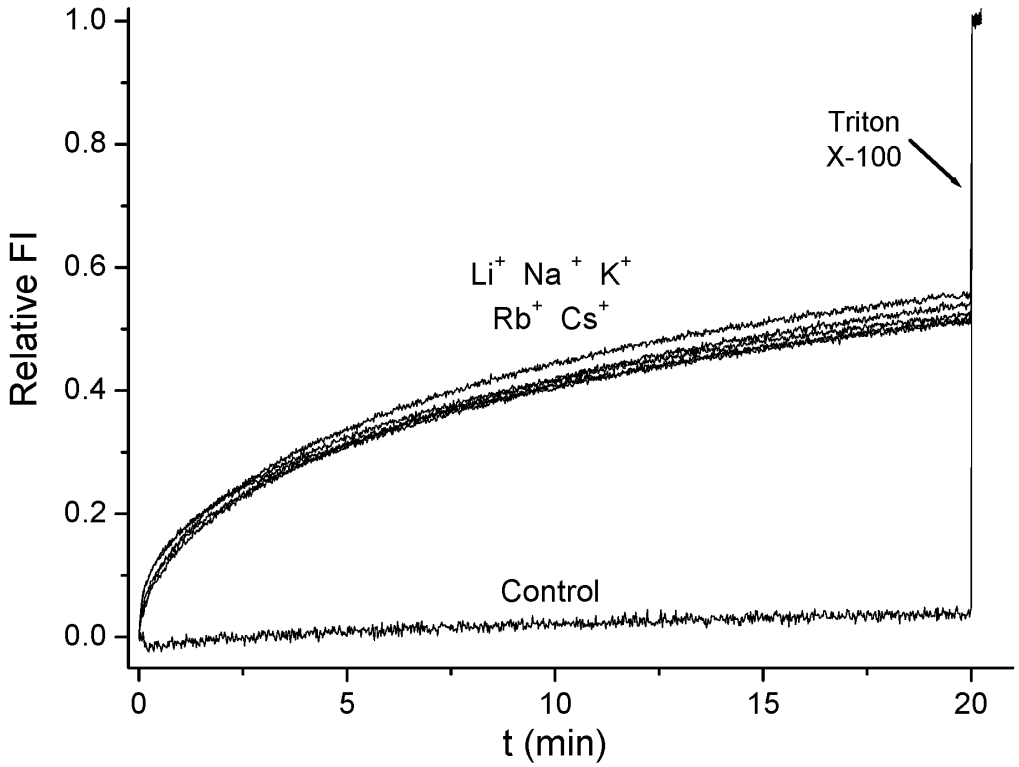


Fig. S7. Discharge of a pH gradient by compound **1** of 3.0 mol% across EYPC-based liposomal membranes (1.33 mM phospholipid) at room temperature. Intravesicular conditions: 0.1 mM pyranine in 25 mM HEPES (pH 7.0, 50 mM MCl); Extravesicular conditions: 25 mM HEPES (pH 8.0, 50 mM MCl) (M = Li, Na, K, Rb and Cs). Ex 460 nm; em 510 nm. The experiment that was conducted in NaCl media and in the absence of compound **1** was used as a control.