

A Fine Line between Unimolecular and Bimolecular Pores Formed from Poly(choloyl) Conjugates Having a Rigid Core

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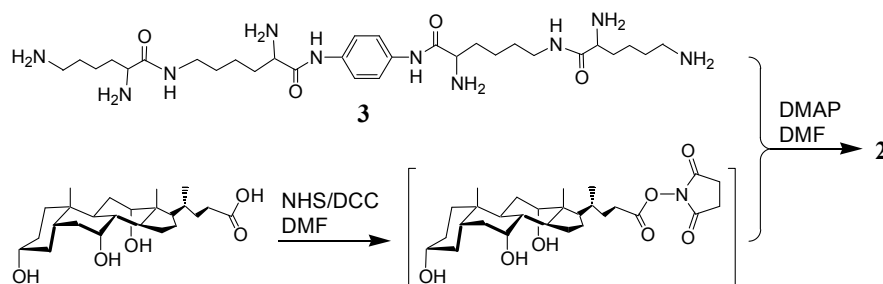
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Supporting Information

General procedures

^1H NMR and $^{23}\text{Na}^+$ NMR spectra were recorded using Bruker 500 and 360 spectrometers, respectively, and deuterium solvents were used as an internal reference in the case of ^1H NMR. MALDI-TOF mass spectrum was recorded on a DE-STR mass spectrometer. Silica gel 60 (Fluka, particle size 0.035~0.070 mm, 220~440 mesh) was used for all column chromatographic purifications. Thin-layer chromatography (TLC) was performed on glass plates that were pre-coated with silica gel containing a fluorescence indicator (Whatman). Detection on TLC was made by use of 10% sulfuric acid in water, iodine, and UV (254 or 365 nm). Compound **3** was prepared according to the reported methods.¹ All the other reagents and chemicals were obtained from commercial sources and used as received unless noted otherwise.

Synthesis of conjugate **2**



To a solution of cholic acid (250 mg, 0.61 mmol) and NHS (80 mg, 0.70 mmol) in anhydrous DMF (2 mL) was added DCC (160 mg, 0.78 mmol). After the mixture was stirred at room temperature for 3 h, a solution of **3** (51 mg, 0.08 mmol) and 4-dimethylaminopyridine (DMAP, 300 mg, 2.46 mmol) in anhydrous DMF (2.0 mL) was added. The reaction mixture was stirred at room temperature for 24 h, and then concentrated and poured into diluted hydrochloride acid (1 M, 200 mL). The resulting precipitates were collected by filtration and purified by chromatography on a silica-gel column, eluted with $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{H}_2\text{O}$ (40/10/1, v/v/v) to give conjugate **2** (35 mg, 14%) having ^1H NMR (500 MHz, CD_3OD , 318 K) δ 7.50 (s, 4H), 4.44 (t, $J = 6.68$ Hz, 2 H), 4.22 (t, $J = 6.69$ Hz, 2 H), 3.93 (s, 6 H), 3.78 (s, 6 H), 3.36-3.34 (m, 6 H), 3.14 (m, 8 H), 2.25-0.91 (m, 192 H), 0.69 (s, 18 H) and MALDI-TOF MS m/z : 2987 ($[\text{M}+\text{Na}]^+$), 3003 ($[\text{M}+\text{K}]^+$).

POPC Vesicle Formation and Na^+/K^+ Transport Measurements

Vesicle formation and Na^+/K^+ transport measurements were conducted according to the reported procedures.² Specifically, 2.5 mL of a 20 mg/mL solution of 1-palmitoyl-2-oleoyl-2-*sn*-glycero-3-phosphocholine (POPC) in chloroform was transferred to a pyrex test tube. The desired amount of conjugate **2** was then added from a stock solution in methanol. While rotating the tube, the organic solvents were removed under a stream of nitrogen, resulting in a thin lipid film. The last traces of solvent were then removed under reduced pressure (25 °C,

12 h, < 0.2 Torr). To the dried film was added 1.0 mL of a 150 mM KCl solution that was 10% in D₂O and 90% in H₂O, and the mixture was vortexed for 1 min. The dispersion was then incubated for 5 min, followed by another 1 min of vortexing and 20 min of incubation at ambient temperature. The sample was subjected to five freeze/thaw cycles (77 K / 325 K), followed by sequential extrusion through a 400 nm Nuclepore membrane (10 times) and a 200 nm membrane (10 times). After extrusion, the dispersion was incubated at room temperature for 1.25 h.

In a quartz NMR tube, 1.5 mL of a 150 mM NaCl solution in 10% D₂O plus 90% H₂O was mixed with 0.3 mL of a shift reagent solution (10 mM DyCl₃ and 30 mM Na₅P₃O₁₀ in 10% D₂O plus 90% H₂O). To this solution was added 0.75 mL of the vesicle dispersion, and the resulting mixture was vortexed for 30 s. ²³Na NMR spectra were recorded continuously at 35°C overnight on a Bruker AMX 360 MHz NMR instrument. Pseudo first-order rate constants were calculated from the change in the percentage of encapsulated Na⁺ as a function of time using a curve-fitting procedure.

Measurement of Single Channel Currents

Single channel currents were observed by using a planar lipid bilayer system described previously.³ Thus, a solution of conjugate **1** in CH₂Cl₂/MeOH (4/1, v/v) (2.0 mg/mL, 1 µL) was mixed with a solution of soybean lecithin (10 mg) in CH₂Cl₂ (100 µL). After removal of the solvents, the obtained residue was dried under vacuum for 30 min. Then, *n*-decane (250 µL) was added to the remaining lipid-conjugate **1** mixture to make a 0.2 wt% premix solution. Bilayers were formed by applying the premix solution to a hole of 0.2-0.3 mm in diameter, precoated with a concentrated lecithin solution in decane (80 mg/mL), and dried prior to the measurement. The channel current measurements were carried out at 25 °C, in a symmetric KCl or NaCl solution buffered with HEPES-Tris (pH 7.2) (Table S1). The conductance was

obtained by plotting the currents against the applied voltages.

DPPC Vesicle Formation and Carboxyfluorescein Release Measurements

Vesicle formation and carboxyfluorescein (CF) release measurements were conducted according to the reported procedures.⁴ Specifically, 2.0 mL of a 25 mg/mL solution of 1-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) in chloroform was transferred to a Pyrex test tube. The desired amount of conjugate **1** or **2** was then added from a stock solution in methanol. While rotating the tube, the organic solvents were evaporated using a stream of nitrogen, resulting in a thin lipid film. The last traces of solvent were then removed under reduced pressure (25 °C, 18 h). To the dried film was added 1.0 mL of a 50 mM CF solution in 10 mM HEPES buffer (pH 7.4) at 60 °C. The resulting mixture was vortexed for 15 s, incubated at 60 °C for 15 s and vortexed 15 s. The dispersion was then incubated at 60 °C for 5 min, vortexed for 15 s twice and incubated at 60 °C for another 20 min. The sample was subjected to five freeze/thaw cycles (77 K / 333 K), followed by extrusion through a 400 nm Nuclepore membrane (10 times) and a 200 nm membrane (10 times) at 60 °C. After extrusion, the dispersion was incubated at room temperature for 1.25 h, the non-entrapped CF was removed via gel filtration (Sephadex G-25, 1×32 cm, eluting with 10 mM HEPES buffer (pH 7.4)).

The vesicle dispersion (0.2 mL) was added to 1.8 mL of a 10 mM HEPES buffer (pH 7.4), which was maintained at the desired temperature on a Perkin Elmer Luminescence Spectrometer LS50B. The appearance of fluorescence intensity was then monitored as a function of time (ex 490 nm, em 514 nm, ex/em 2.5 nm/2.5 nm). After 60 min, 50 µL of 5

wt% Triton X-100 in 10 mM HEPES buffer (pH 7.4) was added. The amount of CF that was released from the vesicles was then calculated using the following equation: %CF release = $(I - I_0)/(I_{\text{total}} - I_0) \times 100$, 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 solution of Triton X-100, respectively.

References:

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2. J. Zhang, B. Jing, S. L. Regen, *J. Am. Chem. Soc.* **2003**, *125*, 13984–13987.
3. W.-H. Chen, K. Nishikawa, S.-D. Tan, M. Yamamura, A. Satake, Y. Kobuke, *Chem. Commun.* **2004**, 872–873.
4. W.-H. Chen, S. L. Regen, *J. Am. Chem. Soc.* **2005**, *127*, 6538–6539.

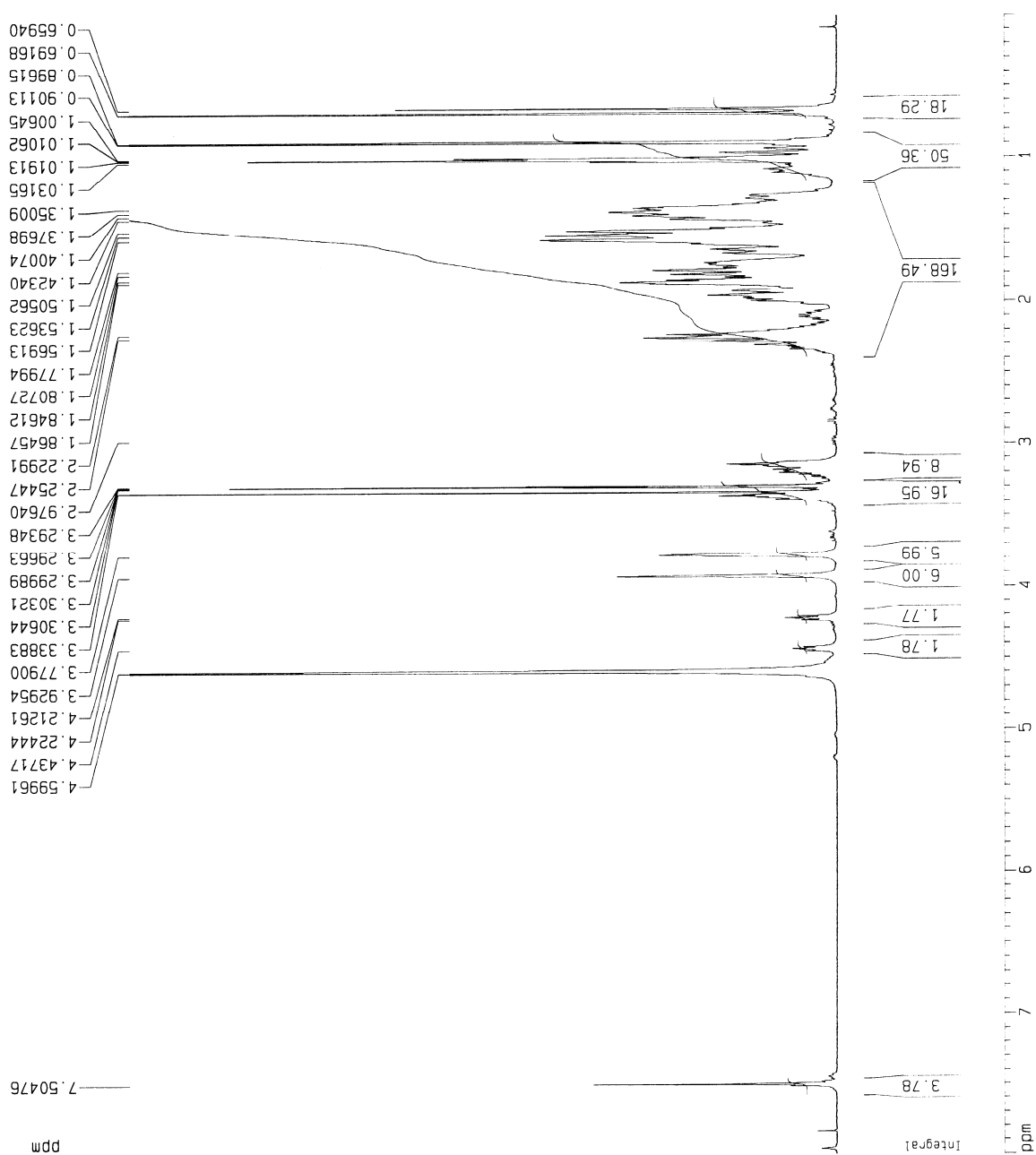


Figure S1. ^1H NMR spectrum (500 MHz) of conjugate **2** in CD_3OD at 318 K.

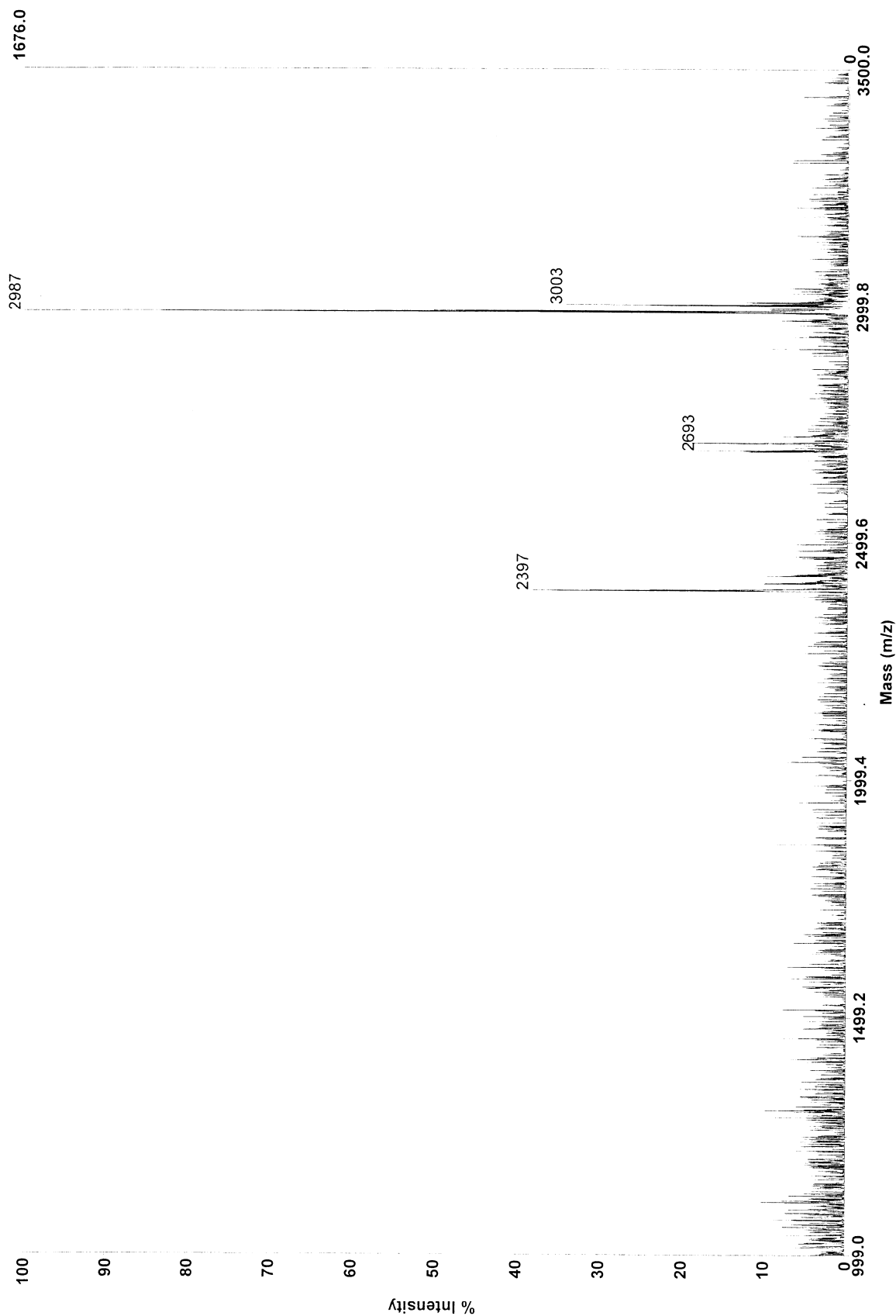


Figure S2. MALDI TOF mass spectrum of conjugate 2.

Table S1. Single channel currents (pA) of conjugate **1** measured at various voltages (mV) ^a

KCl		NaCl	
Voltage	Current	Voltage	Current
+126.5	+1.00	+126.2	+0.63
+116.6	+0.91	+114.3	+0.58
+99.1	+0.74	+101.7	+0.56
-99.1	-0.97	+83.3	+0.42
-116.3	-1.16	+71.4	+0.37
-126.5	-1.20	-71.4	-0.53
		-83.3	-0.55
		-98.4	-0.68
		-116.5	-0.78
		-126.2	-0.84

^a In a symmetric KCl or NaCl solution (0.5 M) buffered with HEPES-Tris (pH 7.2).

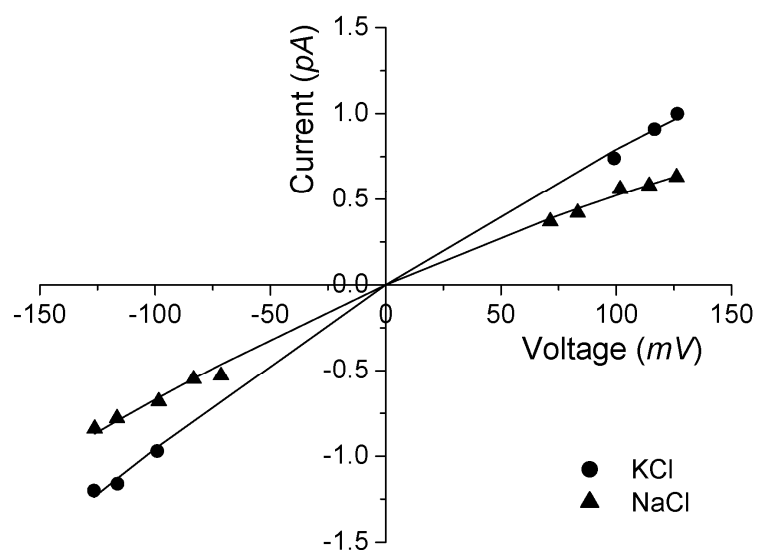


Figure S3. Current–voltage plots of conjugate **1** in symmetric KCl and NaCl solutions (0.5 M) at pH 7.2.