Artificial Ion Channels Showing Rectified Current Behavior

Chigusa Goto,[†] Mika Yamamura,[†] Akiharu Satake,[‡] and Yoshiaki Kobuke*[,†,‡]

Contribution from CREST, Japan Science and Technology Corporation (JST), and Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0101, Japan

Received March 22, 2001. Revised Manuscript Received July 13, 2001

Abstract: Voltage-dependent artificial ion channels **3** and **4** were synthesized. Two cholic acid derivatives were connected through a *m*-xylylene dicarbamate unit at 3-hydroxyl groups. Asymmetries were introduced by terminal hydrophilic groups, carboxylic acid and phosphoric acid for **3** and hydroxyl and carboxylic acid for **4**. Under basic conditions, these headgroups in **3** and **4** are expected to be dissociate into −1/−2 (pH 8.2) and 0/−1 (pH 7.2), respectively. Single ion channel properties were examined by a planar bilayer lipid membrane method under symmetrical 500 mM KCl at pH 8.2 or 7.2. When **3** and **4** were introduced into the bilayer membrane under application of positive voltage (a positive-shift method), the current values at positive applied voltage were larger than the corresponding ones at the negative applied voltage. The current−voltage plots were fitted by curves through a zero point to show clear rectification properties. The direction of rectification could be controlled by positive- or negative-shift methods. Vectorial alignment of terminal headgroup charges by the voltage-shift incorporation is essential for giving voltage-dependent rectified ion channels.

Introduction

Ion channels are molecular ionic devices which facilitate large fluxes of ions across the biological membrane to generate action potentials as signals in brain and nerve systems. Commonly observable current such as 1 pA corresponds to the passage of $\sim 9 \times 10^6$ ions·s^{-1} through a single molecule of ion channel. Even in such enormous dynamic processes, ionic species and magnitude of the flux are strictly regulated by sophisticated design of the selectivity filters and conducting pathways of the ion.[1] Artificial ion channels have been extensively studied to mimic such important biological modes of signal transduction.[2] Therefore, ion conducting pathways have been successfully developed to facilitate the ionic conduction across the bilayer membrane, which otherwise affords high energy barriers for the entry and passage of such highly hydrophilic substrates. Measurement of single channel currents revealed exactly the same behaviors as natural ion channels in view of constant ionic currents with frequent open−closed transitions and a proportional increase as a function of the applied voltages and others. Some of the artificial channels mimicked successfully ion selectivities such as K^+/Cl^-, K^+/Na^+, or others.[3]

However, if one neglects all the pioneering efforts to develop innovative and original ion channels, these artificial channels are regarded as merely a simple ion-conducting pore unless any appropriate gating functions are installed. The introduction of the function to open, shut down, or regulate the overall flux or direction may have utmost importance in the view not only of understanding the core biological function but also of designing future ionics devices. Voltage gating is one of the fundamental tactics to control the overall permeability of ion channels by use of membrane potentials. Channel gates are opened or closed above or below the critical potential.[4] Alamethicin, an antibiotic α-helical nonadecapeptide, forms a voltage-dependent ion channel in artificial[5] and biological[6] membranes. The role of dipoles by directionally aligned hydrogen bonds along the α-helical axes has been claimed as the source of voltage-dependency[7] and further supported by the synthesis of a voltage-dependent ion channel from aligned bundle helices.[8] The aligned α-helical bundles is one of the ideas to introduce the function of voltage dependence. And what else is possible? Voltage-dependent Na^+ channel is believed to be operated by the response of ammonium charges embedded in the membrane to the external voltage change. It is important to mimic several ways of voltage-dependent channel functions to fully understand gating phenomenon and the future development of artificial

[†] CREST, Japan Science and Technology Corporation (JST).

[‡] Graduate School of Materials Science, Nara Institute of Science and Technology.

(1) Hille, B. *Ionic Channels of Excitable Membranes*, 2nd ed.; Sinauer Associates, Inc.: Sunderland, MA, 1992.

(2) Reviews: (a) Gokel, G. W.; Murillo, O. *Acc. Chem. Res.* **1996**, *29*, 425. (b) Kobuke, Y. In *Advances in Supramolecular Chemistry*; Gokel, G. W., Ed.; JAI Press, Inc.: Greenwich, CT, 1997, Vol. 4, p 163. (c) Fyles, T. M.; Straaten-Nijenhuis, W. F. V. In *Comprehensive Supramolecular Chemistry*; Reinhoudt, D. N., Ed.; Elsevier Science Ltd.: Oxford, 1996, Vol. 10, p 53. (d) Voyer, N. *Top. Curr. Chem.* **1996**, *184*, 1. More recent artificial ion channels have been reported: (e) Murillo, O.; Suzuki, I.; Abel, E.; Murray, C. L.; Meadows, E. S.; Jin, T.; Gokel, G. W. *J. Am. Chem. Soc.* **1997**, *119*, 5540. (f) Voyer, N.; Potvin, L.; Rousseau, E. *J. Chem. Soc., Perkin Trans. 2*, **1997**, 1469. (g) Clark, T. D.; Buehler, L. K.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 651. (h) Merritt, M.; Lanier, M.; Deng, G.; Regen, S. *J. Am. Chem. Soc.* **1998**, *120*, 8494. (i) Qi, Z.; Sokabe, M.; Donowaki, K.; Ishida, H. *Biophys. J.* **1999**, *76*, 631. (j) Dieckmann, G. R.; Lear, J. D.; Zhong, Q.; Klein, M. L.; DeGrado, W. F.; Sharp, K. A. *Biophys. J.* **1999**, *76*, 618. (k) Otto, S.; Osifchin, M.; Regen, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 7276. (l) Otto, S.; Osifchin, M.; Regen, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 10440. (m) Fritz, M. G.; Walde, P.; Seebach, D. *Macromolecules* **1999**, *32*, 574. (n) Kobuke, Y.; Nagatani, T. *Chem. Lett.* **2000**, 298. (o) Kobuke, Y.; Ohgoshi, A. *Colloids Surf., A* **2000**, *169*, 187. (p) Baumeister, B.; Sakai, N.; Matile, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 1955. (q) Renkes, T.; Schäfer, H. J.; Siemens, P. M.; Neumann, E. *Angew. Chem., Int. Ed.* **2000**, *39*, 2512. (r) Schrey, A.; Vescovi, A.; Knoll, A.; Rickert, C.; Koert, U. *Angew. Chem., Int. Ed.* **2000**, *39*, 900. (s) DiGiorgio, A. F.; Otto, S.; Bandyopadhyay, P.; Regen, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 11029. (t) Espínola, C. G.; Pérez, R.; Martín, J. D. *Org. Lett.* **2000**, *2*, 3161. (u) Bandyopadhyay, P.; Zhang, V. L.; Sawko, J. A.; Regen, S. L. *J. Am. Chem. Soc.* **2000**, *122*, 12888.

(3) (a) Kobuke, Y.; Ueda, K.; Sokabe, M. *J. Am. Chem. Soc*. **1992**, *114*, 7618. (b) Tanaka, Y.; Kobuke, Y.; Sokabe, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 693. (c) Yoshino, N.; Satake, A.; Kobuke, Y. *Angew. Chem., Int. Ed.* **2001**, *40*, 457. (d) Kobuke, Y.; Nagatani, T. *J. Org. Chem*. **2001**, *66*, 5094.

(4) North, R. A. *Handbook of Receptors and Channels, Ligand- and Voltage-Gated Ion Channels*; CRC Press: Florida, 1995.

(5) (a) Mueller, P.; Rudin, D. O. *Nature* **1968**, *217*, 713. (b) Goldon, L. G. M.; Haydon, D. A. *Biochim. Biophys. Acta* **1972**, *255*, 1014. (c) Eisenberg, M.; Hall, J. E.; Mead, C. A. *J. Membr. Biol.* **1973**, *14*, 143.

(6) Sakmann, B.; Boheim, G. *Nature* **1979**, *282*, 336.

10.1021/ja010761h CCC: $20.00
Published on Web 11/10/2001

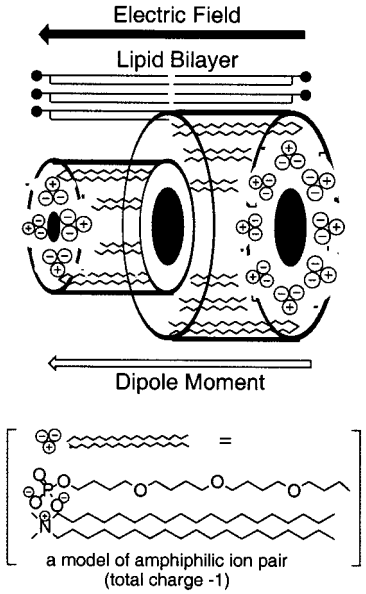


Figure 1. Plausible structure of voltage-dependent ion channel by a phosphoric oligoether and dialkyldimethylammonium pair.

ionics devices. However, there are only a few examples of synthetic voltage-dependent ion channels at present.[9] We have previously reported a prototype design of artificial voltage-dependent ion channels.[9a,b] Hydrophilic oligoether and hydrophobic dialkyl chains were combined to give an amphiphilic membrane-insertible component. To make these components compatible with lipids, ionic headgroups were required, and therefore, dianionic phosphoric monoester and ammonium groups were attached to afford a net negatively charged headgroup (Figure 1). When these lipids were recognized by the membrane components to assemble into a half ion channel, this half channel set should have certain negative charges at the lipid surface. When two half channels are assembled in the membrane to form a tail−tail dimer, the charge numbers are different in general, and the resulting full ion channel has a net dipole moment across the membrane. The single channel current measurements elucidated interesting behavior of typical voltage gating, with the open and closed probabilities being varied depending on the applied voltage. We have been interested in the mechanism of voltage gating and tried to establish structure−function relationships. However, it was difficult to control especially the orientation of each dipole moment of the single channel by using half channel approaches.[10] Recently, we have synthesized transmembrane ion channels **1** and **2** (Figure 2) through linking two amphiphilic cholic acid dimethyl ether by biscarbamate.[3d] Compounds **1** and **2** showed stable single ion channel currents and long-lasting open states, and further, the range of conductance was relatively narrow. Therefore, stable single ion channel with the voltage-dependent property was reasonably anticipated by introducing a membrane spanning dipole moment into the channel molecule. Now, we have synthesized two asymmetric transmembrane cholic acid channels **3** and **4** (Figure 2) and examined their voltage-dependent properties.

Results and Discussion

Two asymmetric transmembrane channels **3** and **4** were designed as candidates for voltage-dependent ion channels. Both compounds are of quasi-C_2 symmetry except the terminal headgroups, which afford electronic asymmetry on their ionization. The headgroups of compounds **3** and **4**, which are carboxylic and phosphoric acid groups, can have charges of −1 and −2, respectively, in fully ionized forms, and a hydroxyl group remains neutral. The molecular lengths of **3** and **4** are fitted to those of bilayer membranes (∼40 Å) based on CPK models, and their rigid and amphiphilic steroidal skeletons are expected to contribute effectively to the construction of stable ion channels.[3c,d] If the orientation of the deprotonated forms of **3** and **4** is controlled vectorially in bilayer membranes by the application of electric field E, their dipole moments μ should be parallel to the applied field, and the resulting supramolecular channels will be illustrated as in Figure 3. Electrostatic repulsions of headgroups are expected to open the channel mouth in the decreasing order $ROP(O)O_2^{2-} > RCO_2^- > ROH$. Comparison of the behavior of **3** and **4** will give information on structure−function relationship.

Compounds **3** and **4** were synthesized as shown in Scheme 1. Bis(cholic acid dimethyl ester) **8** was prepared according to our previous report.[3d] Thus, the 3-hydroxyl group in cholic acid was selectively protected as a tetrahydropyranyl (THP) ether to give **5**. Treatment of methyl iodide with **5** in the presence of NaH followed by deprotection of the THP group afforded cholic acid dimethyl ether **7** with a free 3-OH group. Condensation of 2 equiv of **7** and *m*-xylylenediisocyanate gave **8**. Partial reduction of one of the terminal ester groups in **8** with $LiBH_4$ gave an asymmetric ester alcohol **9** in 47% yield with recovery of starting diester **8** (41%). Treatment of alcohol **9** with $(Cl_3CCH_2O)_2P(O)Cl$ and pyridine produced phosphoric ester **10**. Selective cleavage of a trichloroethyl group with Zn(Cu) in DMF at 50 °C followed by hydrolysis of the carboxylic ester group with 1N NaOH in MeOH gave **3** in 76% yield (2 steps). On the other hand, hydrolysis of ester alcohol **9** with 1N NaOH in MeOH gave **4** in 89% yield.

Single ion channel currents were measured by the planar bilayer lipid membrane method. To align the molecular orientation as shown in Figure 3, the channel-forming process should be controlled. Therefore, preparation of bilayer membrane was performed as follows: (1) A premix solution of soybean lecithin and bis(cholic acid ether) **3** or **4** (0.2 wt %) in a mixture of $CHCl_3$ and *n*-decane (1:5) was prepared. The premix solution was applied to only a cis side[11] of the hole in the partition separating two aqueous chambers. Triangular voltage ramps were applied in the range ±10 mV (3 Hz) at the initial potential of +50 mV (positive-shift method). This process was necessary for full expansion of the bilayer membrane. Normally, this process had been undertaken at the initial potential of 0 mV. Under positive-shift method conditions, more negative functional groups were expected to align to the cis side (the applied voltage side) because of the electrostatic interaction.

After incorporation of **3** to a lipid bilayer membrane by a positive-shift method, stable single channel currents were observed at various applied voltages under conditions of

(7) (a) Hol, W. G. J. *Prog. Biophys. Mol. Biol.* **1985**, *45*, 149. (b) Wooley, G. A.; Epand, P. M.; Kerr, I. D.; Sansom, M. S. P.; Wallace, B. A.; *Biochemistry* **1994**, *33*, 6850. (c) Matsubara, A.; Asami, K.; Akagi, A.; Nishino, N. *Chem. Commun.* **1996**, 2069. (d) Wooley, G. A.; Biggin, P. C.; Schultz, A.; Lien, L.; Jaikaran, D. C. J.; Breed, J.; Crowhurst, K.; Sansom, M. S. P. *Biophys. J.* **1997**, *73*, 770.

(8) Lear, J. D.; Schneider, J. P.; Kienker, P. K.; DeGrado, W. F. *J. Am. Chem. Soc.* **1997**, *119*, 3212.

(9) a) Kobuke, Y.: Ueda, K.: Sokabe, M. *Chem. Lett.* **1995**, 435. (b) Kobuke, Y.; Morita, K. *Inorg. Chim. Acta* **1998**, *283*, 167. (c) Fyles, T. M.; Loock, D.; Zhou, X. *J. Am. Chem. Soc.* **1998**, *120*, 2997.

(10) In the case of these channels, the conductance and the activation potential varied in each experimental run in the range 20 to 150 pS and −75 to +100 mV, respectively.

(11) Voltage was applied to the cis side, and the other side, trans, was connected to the ground.

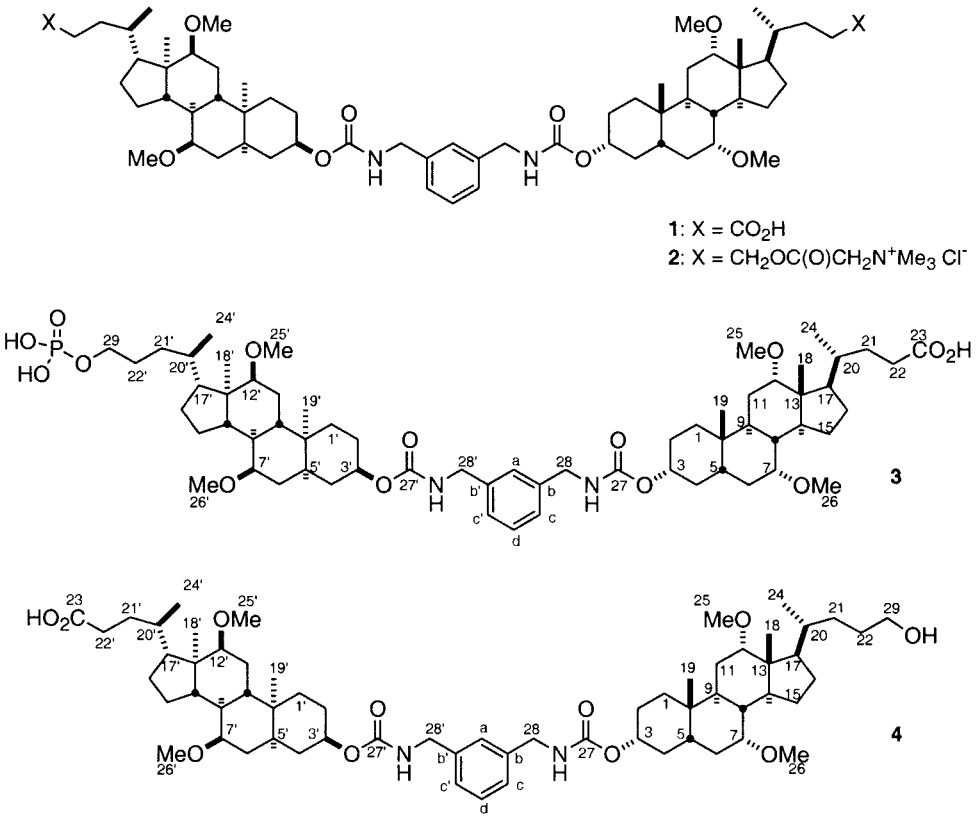


Figure 2. Transmembrane bis(cholic acid) channels **1**, **2**, **3**, and **4**.

symmetrical 500 mM KCl, pH 8.2 adjusted by 2-amino-2-hydroxymethyl-1,3-propanediol and HEPES. At pH 8.2, carboxylic acid groups are expected to be fully deprotonated, and phosphoric acid groups are composed of approximately 91% dianionic and 9% monoanionic forms, if the pK_a values measured in bulk aqueous solution are applied.[12] Figure 4 shows typical records of single channel currents of the same experimental run at +106 mV, +81 mV, −106 mV, and −82 mV. Comparison of absolute current values at +106 mV (1.04 pA) and −106 mV (−0.83 pA), and +81 mV (0.79 pA) and −82 mV (−0.58 pA) shows that the current values at the positive applied voltage are larger than the corresponding ones at the negative applied voltage. The current−voltage relationships of several experimental runs were illustrated in Figure 5. They were fitted by curves through a zero point, and clear rectification function of **3** was observed. To confirm the effect of bulk pH on the dissociation behavior of carboxylic and phosphoric terminals, *I*−*V* plots were evaluated also at pH 9.5 (data points F and G in Figure 5). No discrimination was detected between data sets obtained at these two different pH values. It may support the above postulate that the phosphoric group is dissociated fully into a dianionic form at pH 8.2 to afford carboxylate (−1) and phosphate (−2) as the main existing species.

As seen in the case of other supramolecular ion channels,[2b,n,o,q,r,3a,d,8,9a,b] different current values were observed at the same applied voltage for different experimental runs. At each experimental run, however, one-to-one corresponding current−voltage observation was always maintained. These are characteristics of *supramolecular* ion channels that are formed by assembling lipid-like amphiphilic components in the membrane. The channel forming process is thought to be as follows: During the expansion process of the membrane, the bis(cholic acid ether) part is introduced inside of the membrane, and phospholipids including channel compounds are finally packed vertically to give planar bilayer membrane. Because the inside of the phospholipid membrane is hydrophobic, amphiphilic bis(cholic acid) derivatives introduced in the membrane are unstable to exist individually. Therefore, some of them aggregate with one another by lateral diffusion in such a face-to-face manner that hydrophilic planes create a pore to pass even metal ions. The final structure of these ion channels depends on several factors, which include amphiphilicity of the channel-forming component and its balance between its hydrophobic and hydrophilic natures, cohesive forces of both the channel and lipid components, acceptability of the pore structure in the membrane, and probably many other factors. Combined factors give rise to the formation of ion channels from different numbers of components. Then, we measure the nature of a *single molecular ion channel* for each run. The current at the same applied voltage, and therefore the conductance, varies not only day by day, but also run by run. Even so, it should be kept in mind that each channel structure is maintained unchanged to record the currents by changing the applied voltages. The single ion channel currents around +100 mV are in the range 0.5−1.1 pA and are similar to those of symmetric bis(cholic acid) derivatives **1** and **2** (0.5−2 pA).[3d]

The channel-forming process by application of triangular voltage ramps is essential for full expansion of bilayer membrane. When the initial voltage is reversed during the expansion process, that is, a negative-shift method,[13] more negative functional groups ($ROP(O)O_2^{2-}$) are expected to align to the trans side. The rectification property turned exactly to the opposite direction by a negative-shift method. Current−voltage

(12) The dissociation behavior of **3** was estimated on the basis of pK_{a2} 7.20 of H_3PO_4.

(13) Triangular voltage ramps were applied in the range ±10 mV (3 Hz) from the initial potential of −50 mV.

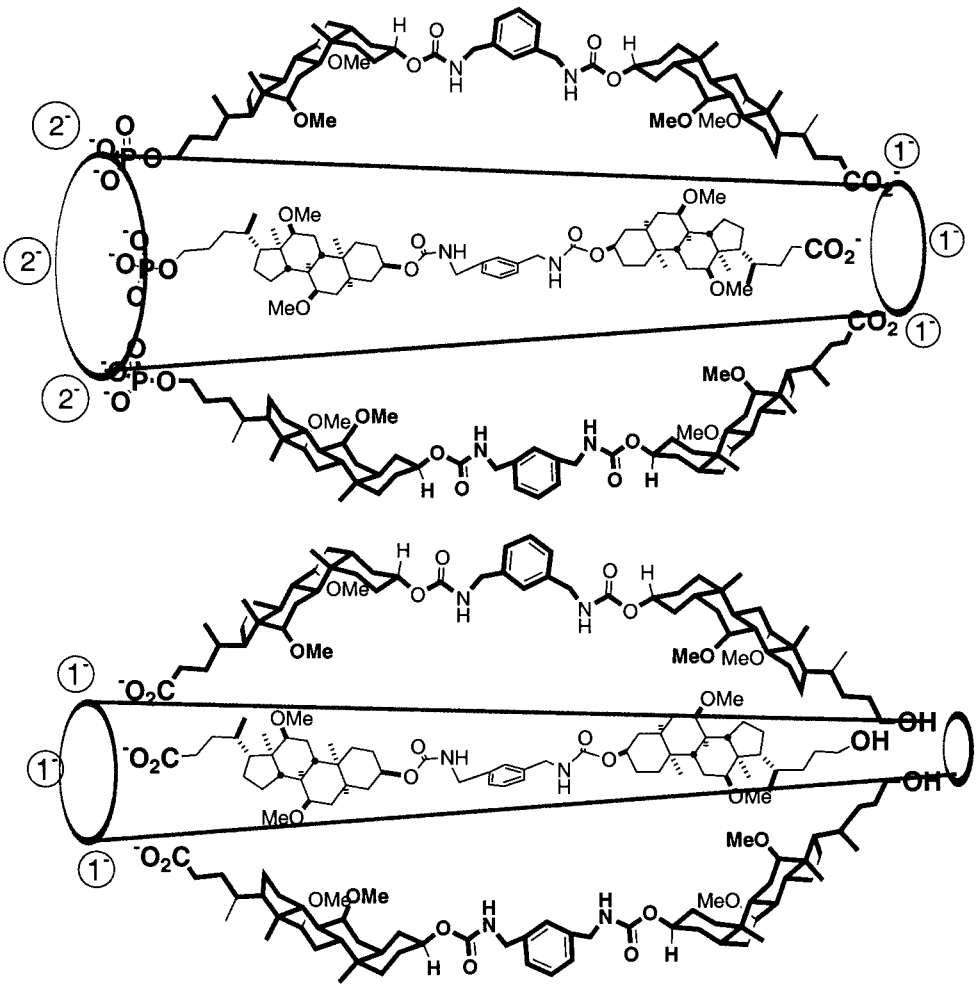


Figure 3. Expected structures of supramolecular ion channels **3** and **4** in lipid bilayer membranes.

plots from two runs were shown in Figure 6. Absolute current values at the positive applied voltage are smaller than the corresponding ones at the negative applied voltage. Fitted solid lines were curved in the opposite direction, compared with those in Figure 5. The symmetry operation at the zero point overlays the result of runs H and I almost exactly to those of B and E, respectively. These results indicate that the alignment process at shifted potential could control the direction of rectification.

For further control experiments, symmetric ion channels of Gramicidin and bis(cholic acid methyl ether) with two carboxylic terminals **1** were measured with the membrane spanning under application of a positive shift voltage of +50 mV. All the plots were analyzed satisfactory by linear functions passing through the origin. No rectification could be observed under the application of asymmetric voltage on membrane spanning, if symmetrical channels are employed. The rectification does result from asymmetric orientation of channel-forming components themselves but not from asymmetric orientation of the lipid components.

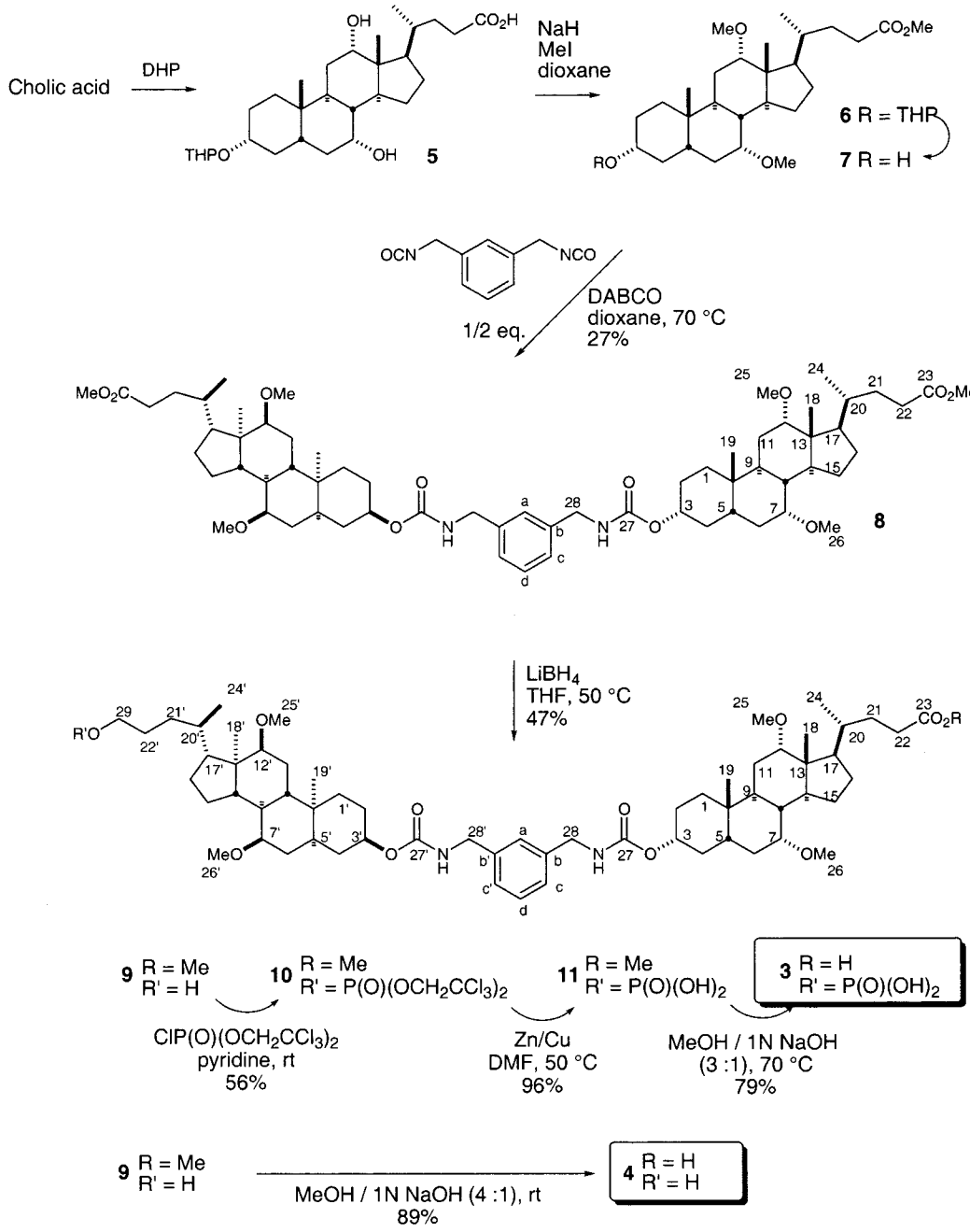
Most synthetic ion channels including symmetric bis(cholic acid) channel **1** are cation selective.[14] Compound **3** is also considered to be cation selective on the basis of the structural similarity to **1**. Therefore, under symmetric 500 mM KCl conditions, the observation of larger ion channel currents at the positive applied voltage (Figure 5) means that potassium ions tend to flow from phosphoric acid ends to the carboxylate ends. The schematic view of the channel appearance was illustrated in Figure 7.

(14) The permeability ratio (P_{K^+}/P_{Cl^-}) for **1** is 11. See ref 3d.

Similarly, channel compound **4** was examined by planar bilayer membrane methods. The channel forming process was performed by a positive-shift method. The current−voltage plots were illustrated in Figure 8. Fine rectification functions with similar curvatures were also observed in the case of **4** except for two experimental runs, denoted by open triangles and filled squares, which could be fitted by linear lines. The range of single ion channel currents (0.6−1.3 pA) around +100 mV is similar to the case of **3**. When the channel-forming process was performed by a negative-shift method, the current−voltage plot curved in the opposite direction (Figure 9).

Fyles reported that a combination of thioacetate (−1) and thioglucose (neutral) headgroups attached to a bismacrocycle was not effective for giving a voltage-dependent ion channel because of the rapid inversion equilibrium transmembrane orientation.[9c] They only succeeded in observing burst type behavior of voltage-dependent multi-ion channels by using a combination of thioacetate (−1) and thiosuccinate (−2) headgroups. However, our results show that the combination of −1 and neutral headgroups is also effective, and the charge difference must be the most fundamental element for voltage-dependent artificial ion channels. To confirm the hypothesis, an electronically symmetric channel by pH control was examined. A premix solution of compound **3** and lipid was applied to the bilayer lipid membrane at pH 5.5. At pH 5.5, it is estimated that 90% of carboxylic acid groups are dissociated and approximately 98% of phosphoric acid groups exist as monoanionic and 2% dianionic. Therefore, charges at both ends of major membrane components are same as −1. The channel

Scheme 1



formation process was performed similarly by a positive-shift method, and single ion channel currents were measured. The current−voltage plots were illustrated in Figure 10. This channel showed a nearly linear current−voltage relationship with a complete loss of the rectification property. The result indicates that electronic asymmetry is given by asymmetric surface charges and that the asymmetry is controlled simply by the bulk pH adjustment.

Current−voltage relationships of **3** at pH 8.2 and **4** at pH 7.2 were almost overlaid (Figures 5 and 8) in all of the applied voltage range. Thus, conductance and rectification behavior was almost same for **3** and **4**. These results support the previous consideration of dissociation behavior of carboxylic and phosphoric groups in the membrane. Because **4** at pH 7.2 must be in the form of carboxylate (−1) and hydroxyl (0) in consideration of the rectification behavior, **3** at pH 8.2 is supported again to be dissociated into phosphate (−2) and carboxylate (−1), as expected from the dissociation constants in the bulk solution.

Further, the conductances of symmetric channels **1** and **2** are similar to those of **3** and **4**, and therefore, the conductance of these channels is mainly determined by the innermembrane components of bis(cholic acid methyl ether), not influenced appreciably by the shape or nature of hydrophilic channel entrances. The change of the rectification property from **3** to **4** was fairly small.

Open durations of channels **3** and **4** were very long (200 ms−4.7 s) at all the applied voltage compared with those observed for alkyl type oligoether half channels.[9a,b] Therefore, the voltage-dependency of open probabilities could not be examined. Such remarkably stable open states are certainly based on the rigid and amphiphilic steroidal skeleton of cholic acid derivatives. The structural stability is also essential to maintain the desired orientation for voltage-dependent channels and to avoid undesired flip−flop phenomenon. Fyles' group reported that the voltage-dependent property disappeared after some repeated triangular potential cycles in the multi-ion channels formed by

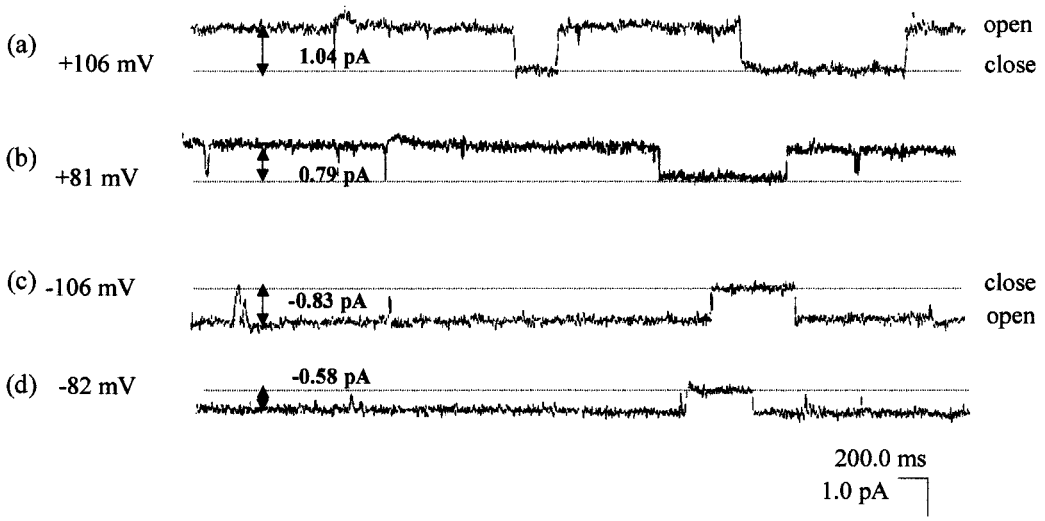


Figure 4. Typical single channel currents by compound **3** at (a) +106mV, (b) +81 mV, (c) −106 mV, and (d) −82 mV, symmetric 500 mM KCl, pH 8.2.

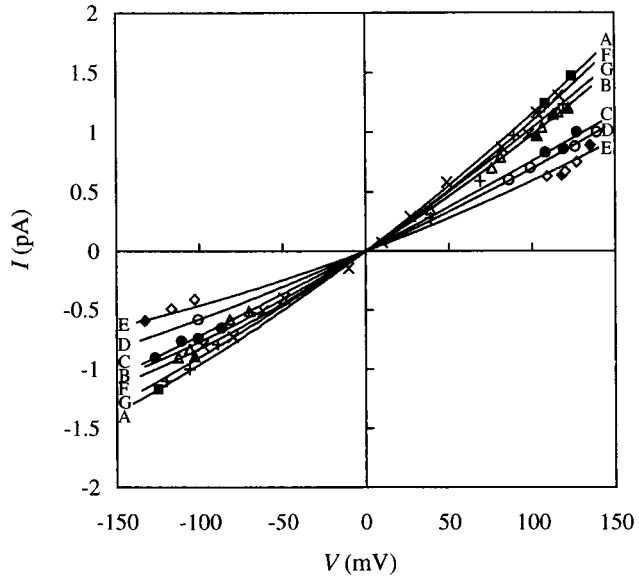


Figure 5. Current−voltage plots of compound **3** prepared by a positive-shift method (symmetric 500 mM KCl) at pH 8.2 (A, ■; B, ▲ and △; C, ●; D, ○; and E,◆ and ◇) and pH 9.5 (F, ×; G, +).

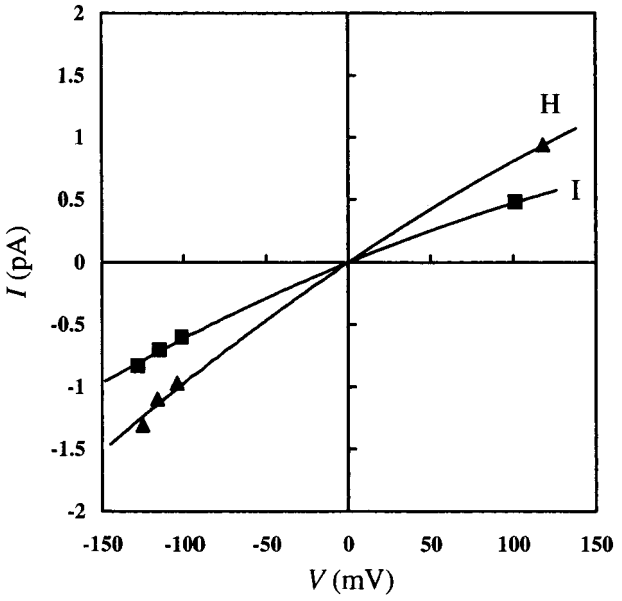


Figure 6. Current−voltage plots of compound **3** prepared by a negative-shift method (symmetric 500 mM KCl, pH 8.2). H, ▲; I, ■.

bismacrocyclic bolaamphiphile.[9c] They explained that the vectorial alignments obtained by the voltage application prior to the measurements were lost easily by the flip−flop mechanism to give random orientation during the measurements. Further, in their cases, voltage-dependent single ion channel currents could not be observed. Under single channel conditions, only burst signals were reported, suggesting that each channel molecule was not stable. In our case, no burst currents were observed, but stable single channels stayed for a long time as usual symmetric ion channels. Neither transition from asymmetric to symmetric current behavior was observed, nor did flip−flop phenomena occur, at least during the measurements.

Concluding Remarks

Two new asymmetric bis(cholic acid) derivatives **3** and **4** were synthesized. Compound **3** has a carboxylic group and a phosphoric acid group at each end, and **4** has a hydroxyl group and a carboxylic group. Both of these ion channels showed stable single ion channel currents with a rectification property. Characteristic properties observed are as follows. (1) When **3** or **4** was incorporated into bilayer lipid membranes, appropriate numbers of components are assembled to produce ion channels characterized by a very stable open state. (2) A vectorial orientation of **3** and **4** having two different charged headgroups could be achieved by the application of biased membrane

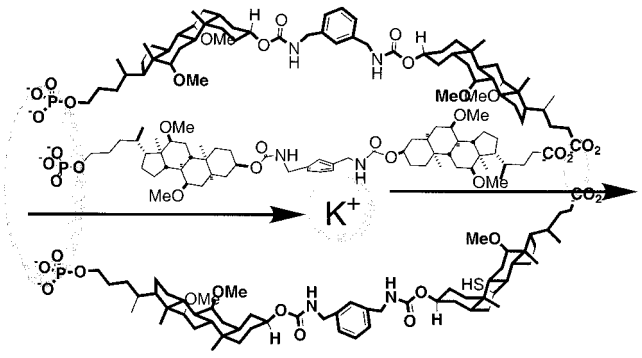


Figure 7. Passage of potassium ion through the ion channel **3**. Potassium ions tend to flow from phosphoric acid ends to the carboxylate ends (see text).

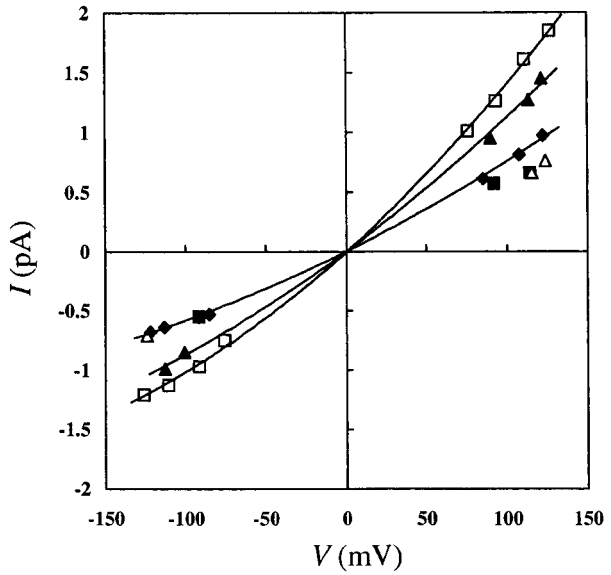


Figure 8. Current−voltage plots of compound **4** prepared by a positive-shift method (symmetric 500 mM KCl, pH 7.2). Points denoted by △ and ■ can be fitted by linear lines.

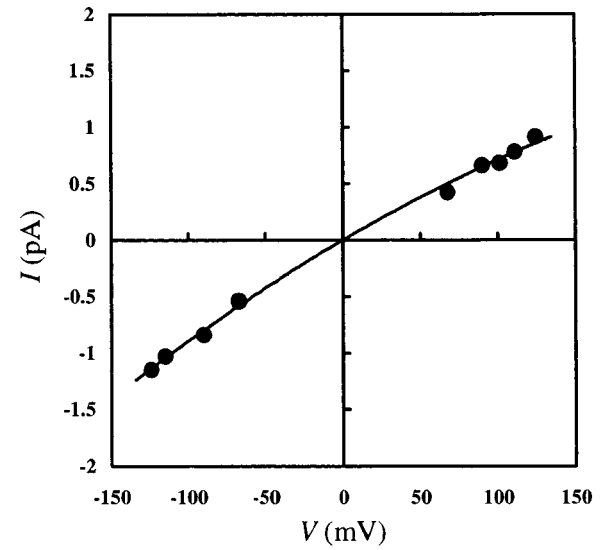


Figure 9. Current−voltage plots of compound **4** prepared by a negative-shift method (symmetric 500 mM KCl, pH 7.2).

voltage during the membrane spanning process, such as positive- or negative-shift methods. If a positive-shift method was employed, more negative headgroups were aligned to the cis side. (3) The orientation of the channel components once formed in the membrane was neither reversed to the opposite direction nor disappeared, even if a larger opposite potential were applied. (4) These oriented ion channels afforded rectified single channel currents. To our knowledge, **3** and **4** are the first stable single ion channels having a rectification property except peptidic channels. The reason is that **3** and **4** contain two fundamental elements for voltage-dependent single ion channels, electrical asymmetry and stability in the bilayer membrane. We believe that **3** and **4** become a prototype of artificial voltage-dependent channels.

Experimental Section

General Procedure. ^{1}H NMR spectra were recorded for $CDCl_3$ or a mixture of $CDCl_3$ and MeOH-d_4 solutions on a JEOL ECP 400, 500, or 600 spectrometer with tetramethylsilane ($\delta = 0$ ppm) as an internal reference. MALDI-TOF mass spectra were measured on a PerSeptive

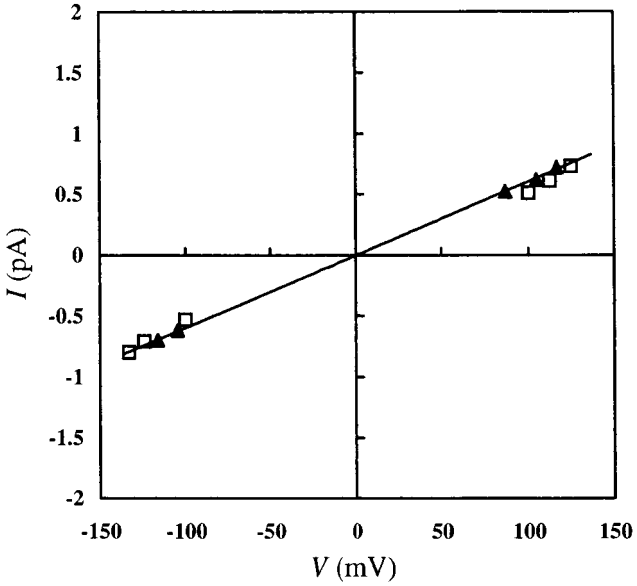


Figure 10. Currents−voltage plots of compound **3** prepared by a positive-shift method (symmetric 500 mM KCl, pH 5.5).

Voyager DE STR through laser ionization with dithranol or α-cyano-4-hydroxycinnamic acid (α-CHCA) as matrixes. ESI-TOF mass spectra were measured on a PerSeptive Mariner by electron spray ionization. Soybean lecithin (type-IIS) was purchased from Sigma Chemical Co. Ltd. and used without purification. Solvents and chemicals were used as received unless otherwise noted. Thin-layer chromatography (TLC) was performed on a glass plate precoated with silica gel (E. Merck Kieselgel 60 F_{254}). Column chromatography was performed with silica gel 60 (E. Merck, particle size 0.063−0.200 mm, 60−230 mesh) or COSMOSIL 75 C_{18}-ONP (reverse phase) purchased from Nacalai Tesque.

Bis[(7,12-dimethoxyl-23-methoxycarbonyl)cholanyl-3-oxy]-*N*,*N*′-xylylene Dicarbamate 8 (Bisester). The synthetic procedure was already reported in ref 3d. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28 (t, 1H, $J = 7.3$ Hz, H_d), 7.21−7.15 (m, 3H), 4.91 (br, 2H, NH), 4.52−4.43 (m, 2H), 4.36−4.24 (m, 4H), 3.65 (s, 6H, CO_2Me), 3.37−3.35 (m, 2H), 3.26 (s, 6H, OMe), 3.21 (s, 6H, OMe), 3.16−3.13 (m, 2H), 2.39−0.98 (m, 48H), 0.91 (d, 6H, $J = 6.7$ Hz, H_{24}), 0.89 (s, 6H, H_{19}), 0.65 (s, 6H, H_{18}). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.9 (C_{23}), 156.5 (C_{27}), 139.3 (C_b), 129.0 (C_d), 126.8 (C_c or C_a), 126.6 (C_c or C_a), 82.2 (C_{12}), 77.2 (C_7), 75.1 (C_3), 56.0 (OMe), 55.9 (OMe), 51.5 (CO_2Me), 46.4 (CH), 46.2 (C), 44.9 (C_{28}), 43.0 (CH), 41.8 (CH), 39.6 (CH), 35.2 (CH), 35.1 (CH_2), 34.6 (C), 34.3 (CH_2), 31.1 (2 carbons, CH_2), 28.0 (CH), 27.8 (CH_2), 27.4 (CH_2), 27.1 (CH_2), 23.2 (CH_2), 22.8 (C_{19}), 22.1 (CH_2), 17.5 (C_{24}), 12.6 (C_{18}). MALDI-TOF (positive mode, matrix: α-CHCA): *m*/*z* calcd for $C_{64}H_{100}N_2O_{12}$, 1088.73; found, 1112.2 $(M + Na)^+$ and 1129.2 $(M + K)^+$.

Alchohol−Ester 9. $LiBH_4$ (2.0 mg, 0.09 mmol) was added to a solution of **8** (196 mg, 0.18 mmol) in 5 mL of dry THF. The mixture was stirred for 3.5 h at room temperature, and the mixture was further stirred at 50 °C for 30 min. After cooling to rt, 1 N HCl solution was added to the reaction mixture to adjust the pH to be acidic, and the reaction mixture was extracted with $CHCl_3$ and dried over $MgSO_4$. After evaporation of the solution, the residue was purified by column chromatography (SiO_2, benzene/AcOEt = 4:1 to 2:1) to yield **9** (90 mg, 47%). The starting material **8** (80 mg, 41%) was recovered. **9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28, (t, 1H, J = 7.3 Hz, H_d), 7.21 − 7.16 (m, 3H), 4.99 (br, 2H, NH), 4.55 − 4.41 (m, 2H), 4.39 − 4.22 (m, 4H), 3.66 (s, 3H, CO_2Me), 3.65 − 3.58 (m, 2H), 3.41 − 3.33 (m, 2H), 3.28 (s, 6H, OMe), 3.22 (s, 6H, OMe), 3.16 − 3.13 (m, 2H), 2.40 − 0.95 (m, 48H), 0.93 (d, 6H, $J = 6.7$ Hz, H_{24}), 0.91 (s, 6H, H_{19}), 0.66 (s, 6H, H_{18}). MALDI-TOF (positive mode, matrix: α-CHCA): *m*/*z* calcd for $C_{63}H_{100}N_2O_{11}$, 1060.73; found, 1084.1 $(M + Na)^+$ and 1100.2 $(M + K)^+$.

Phosphoric Triester−Carboxylic Ester 10. Bis(2,2,2-trichloroethyl)phosphorochloridate (0.38 g, 1 mmol) was added to a solution of

9 (0.11 g, 0.1 mmol) in dry pyridine (0.8 mL). The mixture was stirred for 3 h at rt. Water was added to the reaction mixture, and the organic layer was extracted twice with $CHCl_3$. After evaporation of the solution, the residue was purified by column chromatography (SiO_2, benzene/AcOEt = 10:1 to 4:1) to give colorless amorphous product **10** (78 mg, 56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29−7.27 (m, 2H, H_a, H_c, $H_{c'}$), 7.21−7.12 (m, 1H, H_a), 4.92 (br, 2H, NH), 4.65−4.56 (m, 4H, H_{31}), 4.52−4.42 (m, 2H, H_3, $H_{3'}$), 4.38−4.24 (m, 4H, H_{28}, $H_{28'}$), 4.23−4.15 (m, 2H, H_{29}), 3.66 (s, 3H, CO_2Me), 3.37−3.33 (m, 2H, H_{12}, $H_{12'}$), 3.27 (s, 6H, H_{25}, $H_{25'}$), 3.21 (s, 6H, H_{26}, $H_{26'}$), 3.15−3.12 (m, 2H, H_7, $H_{7'}$), 2.39−2.14 (m, 4H), 2.09−1.95 (m, 4H), 1.95−1.86 (m, 2H), 1.86−1.31 (m, 26H), 1.26−1.10 (m, 8H), 1.10−0.96 (m, 4H), 0.92 (d, 6H, J = 6.7 Hz, H_{24}, $H_{24'}$), 0.91 (s, 6H, H_{19}, $H_{19'}$), 0.66 (s, 6H, H_{18}, $H_{18'}$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.8 (C_{23}), 156.4 (C_{27}), 139.1 (C_b), 128.9 (C_d), 126.7 (C_c or C_a), 126.5 (C_c or C_a), 94.6 (C_{32}), 82.0 (C_{12}, $C_{12'}$), 77.1 (3 carbons, C_{31}, C_7, $C_{7'}$), 75.0 (C_3, $C_{3'}$), 70.1 (C_{29}), 55.9 (C_{26}), 55.7 (C_{25}), 51.4 (C_{30}), 46.3, 46.09, 46.07, 44.8 (C_{28}), 42.9, 41.6, 39.4, 35.1, 34.9, 34.5, 34.1, 31.3, 30.9, 29.7, 27.9, 27.7, 27.5, 27.3, 27.0, 26.71, 26.66, 23.1, 22.7 (C_{19}, $C_{19'}$), 22.0, 17.6 (C_{24}), 17.4 ($C_{24'}$), 12.5 (C_{18}, $C_{18'}$). MALDI-TOF (positive mode, matrix: α-CHCA): *m*/*z* calcd for $C_{67}H_{103}Cl_6N_2O_{14}P$, 1400.53; found, 1423.6 $(M + Na)^+$ and 1439.2 $(M + K)^+$.

Phosphoric Monoester−Carboxylic Ester 11. Acetylacetone (52 μL, 0.51 mmol) and zinc−copper complex[15] (66 mg, 0.01 mmol) were added to a solution of **10** (71.2 mg, 0.051 mmol) in dry DMF (1 mL), and the mixture was stirred at 50 °C for 16 h. 1 N HCl solution was added to the reaction mixture to adjust to pH 1, and the organic layer was extracted with $CHCl_3$. Solvent was removed under reduced pressure below 35 °C. The residue was purified by reverse phase column chromatography [COSMOSIL 75C_{18}-ONP, 1.5 Φ × 4 cm height; H_2O/MeOH (gradiently) 1:0 (50 mL), 7:3 (20 mL), 3:7 (20 mL), 0:1 (50 mL)] to give product **11** (53 mg, 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.81 (br, 2H, POH), 7.25 (br, 1H, H_d), 7.19 (br, 3H, H_a, H_c, $H_{c'}$), 5.02 (br, NH), 4.52−4.43 (m, 2H, H_3, $H_{3'}$), 4.38−4.24 (m, 4H, H_{28}, $H_{28'}$), 3.98−3.90 (m, 2H, H_{29}), 3.65 (s, 3H, CO_2Me), 3.39−3.35 (m, 2H, H_{12}, $H_{12'}$), 3.27 (s, 6H, H_{25}, $H_{25'}$), 3.21 (s, 6H, H_{26}, $H_{26'}$), 3.16−3.13 (m, 2H, H_7, $H_{7'}$), 2.40−2.14 (m, 4H), 2.10−1.96 (m, 4H), 1.96−1.63 (m, 16H), 1.63−1.12 (m, 18H), 1.12−0.95 (m, 4H), 0.95−0.84 (m, 12H, H_{19}, $H_{19'}$, H_{24}, $H_{24'}$), 0.66 (s, 6H, H_{18}, $H_{18'}$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.8 (C_{23}), 156.5 (C_{27}, $C_{27'}$), 139.1 (C_b), 128.9 (C_d), 126.7 (C_c), 126.5 (C_a), 82.3 (C_{12}), 82.2 ($C_{12'}$), 77.5 (C_7, $C_{7'}$), 75.0 (C_3, $C_{3'}$), 68.0 (C_{29}), 55.9 (C_{26}, $C_{26'}$), 55.8 (C_{25}, $C_{25'}$), 51.5 (C_{30}), 46.6, 46.3, 46.1, 44.8 (C_{28}, $C_{28'}$), 42.9, 41.6, 39.4, 35.1, 34.9, 34.5, 34.1, 31.6, 31.4, 30.9, 27.9, 27.7, 27.3, 27.0, 23.1, 22.7 (C_{19}, $C_{19'}$), 22.6, 22.0, 17.7 (C_{24}, $C_{24'}$), 17.4, 14.1, 12.5 (C_{18}, $C_{18'}$). ^{31}P NMR (202 MHz, $CDCl_3$, external ref H_3PO_4 in $CDCl_3$) δ 2.73.

Phosphoric Monoester−Carboxylic Acid 3. Ester **11** (53 mg, 0.0465 mmol) was dissolved in hot MeOH (3 mL), and 1 N NaOH solution (1 mL) was added to the solution. The mixture was stirred at 70 °C for 40 h, and 1 N HCl solution was added to the reaction mixture to adjust to pH 1. All solvent was removed under reduced pressure, and the residue was purified by column chromatography [Cosmo seal 75C_{18}-ONP, 1.5 Φ × 4 cm height; H_2O/MeOH (gradiently) 1:0 (50 mL), 1:1 (10 mL), 3:7 (10 mL), 0:1 (50 mL)] to give product **11** (33 mg, 64%). ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD (4:1)) δ 7.18 (t, 1H, H_d, J = 7.3 Hz), 7.08 (d, 2H, H_c, J = 7.3 Hz), 7.07 (s, 1H, H_a), 4.38−4.31 (m, 2H, H_3, $H_{3'}$), 4.25−4.13 (m, 4H, H_{28}, $H_{28'}$), 3.88−3.79 (m, 2H, H_{29}), 3.34−3.26 (m, 2H, H_{12}, $H_{12'}$), 3.17 (s, 6H, H_{25}, $H_{25'}$), 3.11 (s, 6H, H_{26}, $H_{26'}$), 3.08−3.04 (m, 2H, H_7, $H_{7'}$), 2.30−2.05 (m, 4H), 2.00−1.86 (m, 4H), 1.86−0.88 (m, 20H), 0.85−0.74 (m, 12H, H_{19}, $H_{19'}$, H_{24}, $H_{24'}$), 0.56 (s, 6H, H_{18}, $H_{18'}$). ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD (4:1)) δ 177.2 (C_{23}), 156.9 (C_{27}, $C_{27'}$), 139 (C_b), 128.3 (C_d), 126.6 (C_c), 126.4 (C_a), 82.2 (C_{12}), 82.1 ($C_{12'}$), 77.5 (C_7, $C_{7'}$), 75.0 (C_3, $C_{3'}$), 67.4 (C_{29}), 55.8 (C_{26}, $C_{26'}$), 55.6 (C_{25}, $C_{25'}$), 46.6, 46.3, 46.1, 46.0, 44.5 (C_{28}, $C_{28'}$), 42.8, 41.6, 39.4, 35.2, 35.0, 34.8, 34.4, 34.2, 31.5, 30.9, 29.6, 27.9, 27.7, 27.4, 27.3, 27.0, 23.1, 22.6 (C_{19}, $C_{19'}$), 22.0, 17.6, 17.3 (C_{24}, $C_{24'}$), 12.4 (C_{18}, $C_{18'}$). ^{31}P NMR (202 MHz, $CDCl_3$, external ref H_3PO_4 in $CDCl_3$) δ 1.11. ESI-TOF (positive mode, Ac_3-β-CD as an internal reference): *m*/*z* calcd for $C_{62}H_{99}N_2O_{14}P$, 1126.68; found, 1127.694 $(M + H)^+$ and 1149.687 $(M + Na)^+$.

Alcohol−Carboxylic Acid 4. 1 N NaOH solution (1 mL) was added to a solution of **9** (90 mg, 0.085 mmol) in MeOH (4 mL), and the mixture was stirred at rt for 18 h. Water was added to the reaction mixture, and 1 N HCl solution was further added to adjust to pH 1. The organic layer was extracted twice with $CHCl_3$, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by column chromatography (SiO_2, $CHCl_3$/MeOH = 1:0 to 25:2) to give colorless amorphous product **4** (80 mg, 89%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28 (t, 1H, J = 7.3 Hz, H_d), 7.20−7.17 (m, 3H, H_a, H_c, $H_{c'}$), 4.92 (br, 2H, NH), 4.51−4.44 (m, 2H, H_3, $H_{3'}$), 4.37−4.25 (m, 4H, H_{28}, $H_{28'}$), 3.66−3.58 (m, 2H, H_{29}), 3.40−3.35 (m, 2H, H_{12}, $H_{12'}$), 3.27 (s, 3H, H_{25} or $H_{25'}$), 3.26 (s, 3H, H_{25} or $H_{25'}$), 3.21 (s, 6H, H_{26}, $H_{26'}$), 3.15−3.12 (m, 2H, H_7, $H_{7'}$), 2.43−0.96 (m, 48H), 0.92 (d, 6H, J = 6.7 Hz, H_{24}, $H_{24'}$), 0.90 (s, 6H, H_{19}, $H_{19'}$), 0.66 (s, 6H, H_{18}, $H_{18'}$). ^{13}C NMR (150 MHz, $CDCl_3$) δ 178.6 (C_{23}), 156.6 (C_{27}), 139.2 (C_b), 129.0 (C_d), 126.8 (C_c or C_a), 126.6 (C_c or C_a), 82.2 (C_{12} or $C_{12'}$), 82.1 (C_{12} or $C_{12'}$), 77.1 (C_7, $C_{7'}$), 75.1 (C_3, $C_{3'}$), 63.7 (C_{29}), 56.0 (C_{26}, $C_{26'}$), 55.9 (C_{25}, $C_{25'}$), 46.7 (CH), 46.4 (CH, 2 carbons), 46.23 (C), 46.19 (C), 44.9 (C_{28}, $C_{28'}$), 43.0 (CH, 2 carbons), 41.8 (CH, 2 carbons), 39.6 (CH, 2 carbons), 35.5 (CH), 35.12 (CH), 35.05 (CH_2, 2 carbons), 34.6 (C, 2 carbons), 34.3 (CH_2, 2 carbons), 31.9 (CH_2), 30.9 (CH_2), 30.8 (CH_2), 29.4 (CH_2), 28.0 (CH, 2 carbons), 27.8 (CH_2, 2 carbons), 27.6 (CH_2), 27.4 (CH_2), 27.1 (CH_2, 2 carbons), 23.2 (CH_2, 2 carbons), 22.8 (C_{19}, $C_{19'}$), 22.2 (CH_2, 2 carbons), 17.8 (C_{24}), 17.5 ($C_{24'}$), 12.6 (C_{18}, $C_{18'}$). ESI-TOF (positive mode, Ac_3-β-CD as an internal reference): *m*/*z* calcd for $C_{62}H_{98}N_2O_{11}$, 1046.72; found, 1047.723 $(M + H)^+$ and 1069.723 $(M + Na)^+$.

Measurement of Single Channel Currents. Single ion channel currents were measured by the planner bilayer lipid membrane method. Details may refer to the previous papers.[3] Soybean lecithin (10 mg) was dissolved in *n*-decane (0.2 mL) in a micro glass tube. To this solution, 0.04 mL of chloroform solution of bis(cholic acid ether) **3** or **4** (0.5 mg/mL) was added. The mixture was sonicated for about 1 min to give a clear solution (0.2 wt % premix solution). The premix solution was applied to only a cis side[11] of a hole precoated with a more concentrated lecithin solution in *n*-decane (80 mg/mL) in a partition separating two aqueous chambers adjusted to appropriate pH values. Next, to align the orientation of channel compounds, two kinds of membrane-expansion processes were performed. Positive-shift method: triangular voltage ramps were applied in the range ±10 mV (3 Hz) at the initial potential of +50 mV. Under the conditions, more negative functional groups were aligned to the cis side (the applied voltage side) because of the electrostatic attraction. Negative-shift method: triangular voltage ramps were applied in the range ±10 mV (3 Hz) at the initial potential of −50 mV. Under the conditions, more negative functional groups were aligned to the trans side (the ground side) because of the electrostatic repulsion. After incorporation of channel compounds, initial data were collected at different positive applied potential in the case of positive shifted method. Then, data were collected in the negative applied potential area. Further, data collection was continued until open−closed phenomena disappeared. In the case of the negative-shift method, data collection was performed in the reverse order. The data storage and analysis were undertaken in a similar way as reported previously.[3]

Acknowledgment. The authors are grateful for helpful discussion by Professor Masahiro Sokabe at Graduate School of Medicine, Nagoya University.

JA010761H

(15) (a) Eckstein, F. *Chem. Ber.* **1967**, *100*, 2236. b) Heuer, M.; Hohgart, K.; Heinemann, F.; Kühne, H.; Dietrich, W.; Grzelak, D.; Müller, D.; Welzel, P.; Markus, A.; Heijenoort, Y. V.; Heijeenoort, J. V. *Tetrahedron* **1994**, *50*, 2029.