Molecular Design and Synthesis of Artificial Ion Channels Based on Cyclic Peptides Containing Unnatural Amino Acids

Hitoshi Ishida,*[,†] Zhi Qi,[‡] Masahiro Sokabe,*[,‡,§] Kiyoshi Donowaki,[∥] and Yoshihisa Inoue[†,∥]

Inoue Photochirogenesis Project, ERATO, Japan Science and Technology, 4-6-3 Kamishinden, Toyonaka, Osaka 560-0085, Japan, Department of Physiology, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan, Cell Mechanosensing Project, ICORP, Japan Science and Technology, 65 Tsurumai, Nagoya 466-8550, Japan, and Department of Molecular Chemistry, Osaka University, Yamada-oka, Suita, Osaka 565-0871, Japan

ishida@inoue.jst.go.jp

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A series of novel cyclic peptides composed of 3 to 5 dipeptide units with alternating natural−unnatural amino acid units, have been designed and synthesized, employing 5-(*N*-alkanoylamino)-3-aminobenzoic acid with a long alkanoyl chain as the unnatural amino acid. All cyclic peptides with systematically varying pore size, shape, and lipophilicity are found to form ion channels with a conductance of ca. 9 pS in aqueous KCl (500 mM) upon examination by the voltage clamp method. These peptide channels are cation selective with the permeability ratio P_{Cl^-}/P_{K^+} of around 0.17. The ion channels formed by the neutral, cationic, and anionic cyclic peptides containing L-alanine, L-lysine, and L-aspartate, respectively, show the monovalent cation selectivity with the permeability ratio P_{Na^+}/P_{K^+} of ca. 0.39. On the basis of structural information provided by voltage-dependent blockade of the single channel current of all the tested peptides by Ca^{2+}, we inferred that each channel is formed from a dimer of the peptide with its peptide ring constructing the channel entrance and its alkanoyl chains lining across the membrane to build up the channel pore. The experimental results are consistent with an idea that the rate of ion conduction is determined by the nature of the hydrophobic alkanoyl chain region, which is common to all the channels.

Introduction

Ion channels are huge membrane proteins which generate electrical and calcium signals in most of the cells including neurons.[1] Synthetic low molecular weight compounds which exhibit ion channel activity have been investigated as models for the native ion channels. These model studies may have two general advantages: (1) We can elucidate the biochemical or biophysical properties of the natural ion channels through the knowledge obtained from the investigation of the ion channel behavior of model compounds, because the comprehensive understanding of the structure and dynamics of the native ion channels seems difficult to achieve at present. (2) We can obtain knowledge to design and construct molecular devices that can convert chemical signals to electrical ones, mimicking the biological signal transduction system.

The most important ion channel model molecules are peptides, which include bioactive peptides, such as antibiotics and toxins,[2−8] and their derivatives.[9−16] This category also includes model peptides employed as the segments in the active site of natural ion channels[17−19] and amphipathic polypeptides.[20] Some of the peptides, e.g., gramicidin, form a single molecular helix, the central pore of which can permeate ions.[2,5,6,9−12] However, most of the peptides, e.g., alamethicin, aggregate to form a barrellike structure, forming a hydrophilic pore in its center in bilayer membranes.[3,4,13−15] In the latter cases, the domain peptides have been gathered by using the template-assembled synthetic protein (TASP) method,[21,22]

* Corresponding authors: E-mail for M. Sokabe: msokabe@med.nagoya-u.ac.jp.
† Inoue Photochirogenesis Project, ERATO.
‡ Nagoya University School of Medicine.
§ Cell Mechanosensing Project, ICORP.
∥ Osaka University.

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and furthermore their function−structure relationships have been investigated by controlling the assembling numbers.[15,22−25] As an interesting example, Ghadiri et al. have reported that cyclic peptides composed of alternating D- and L-amino acids form a peptide nano tube through which ions can be transported.[26−29] Thus, the peptide ion channels build up a hydrophilic pore in bilayer membranes by employing the peptide backbone or hydrophilic side chains of the amino acid residues.

Ion channels do not necessarily have peptide structures. Until now, a large numbers of non-peptide ion channels have been designed and synthesized by organic chemists who expect the application of molecular devices which convert chemical signals into electric ones.[30,31] These artificial ion channels mostly bear either crown ether rings[32−43] or polyethers[44−48] or both of them[49−57] as the hydrophilic sites. For example, there have been reported artificial ion channels with crown ethers appended to peptide,[32−34] poly(isocyanide),[42,43] and alkyl backbones,[35−41] although the first one is not fully nonpeptidic. Hydrophilic polyethers, which are modified with hydrophobic sterol[47] or phospholipids,[48] have also been synthesized as artificial ion channels. To assemble polyether chains to form a hydrophilic pore, the polyethers possessing anionic headgroups are mixed with hydrophobic long alkyl compounds with cationic headgroups to form an ion pair, which indeed function as artificial ion channels.[44−46] Combinations of crown ether and polyether also behave as artificial ion channels.[49−57] Furthermore, polyol with rigid, hydrophobic oligo(*p*-phenylene) skeleton[58] and polyesters such as poly(2-ethylacrylic acid)[59] or poly-(3-hydroxybutyrate)[60] have been reported to function as ion channels which do not employ either the crown ethers or polyethers.

From the foregoing studies mentioned above, the ion-channel-forming molecules are considered in general to form a hydrophilic pore in bilayer membranes. However, some cyclic compounds composed of only hydrophobic long alkyl chains have been reported to exhibit ion channel activity. Tabushi et al. reported that β-cyclodextrin modified by four long alkyl chains transports cobalt(II) ions across a bilayer membrane, although this molecule was not fully demonstrated to be an ion channel or a carrier because the transport ability was just evaluated by the amounts of cobalt(II) ions transported from inside of liposome to outside.[61] Tanaka et al. demonstrated by single channel recording that a cyclic resorcinol tetramer with long alkyl chains acts as a half channel with K^+ selectivity.[62] They also implied that the K^+ selectivity appeared in the cyclic resorcinol tetramer acting as a selective filter. The action of such an ion channel bearing a hydrophobic pore is very interesting in relation to that of natural ion channel proteins having hydrophobic pore region: for example, the uncharged and very hydrophobic M2 segments of the nicotinic acetylcholine receptor (nAChR), ••• MTLSISVLLSLTVFLLVIV •••.[63]

We have proposed a strategy for designing novel functional peptides by utilizing a rigid unnatural amino acid such as 3-aminobenzoic acid.[64−68] Introduction of the rigid unnatural amino acid into the target peptide affords a more defined structure. Thus, the present strategy provides us with a tool for controlling the function of

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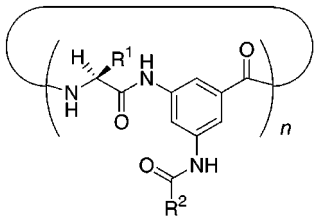


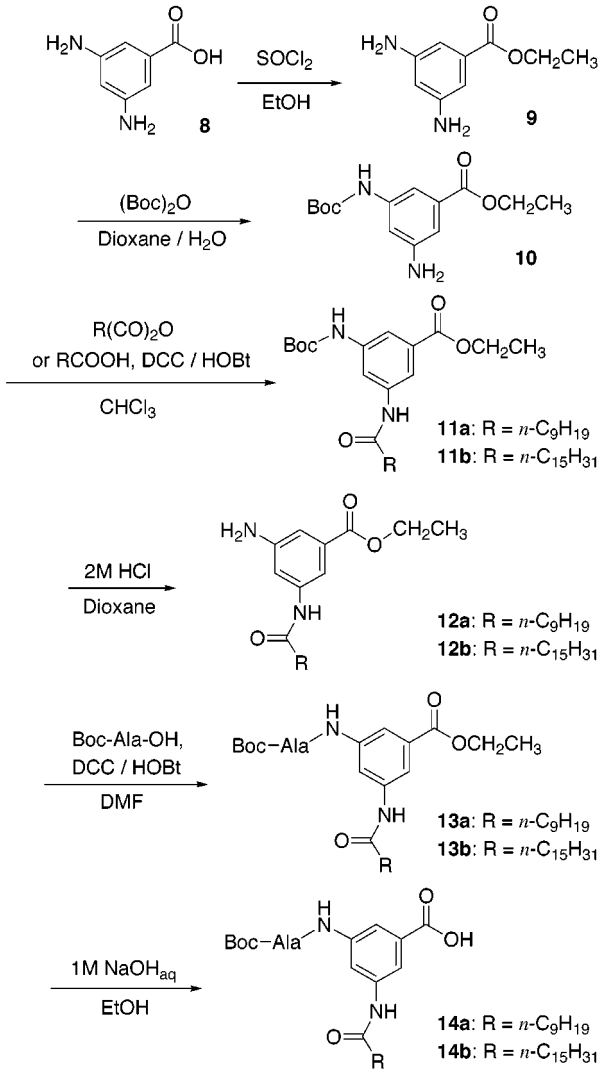
	R^1 (Amino Acid)	R^2	*n*
$(AC_{10})_3$ (**1**)	-CH_3 (L-Ala)	*n*-C_9H_{19}	3
$(AC_{10})_4$ (**2**)	-CH_3 (L-Ala)	*n*-C_9H_{19}	4
$(AC_{16})_4$ (**3**)	-CH_3 (L-Ala)	*n*-$C_{15}H_{31}$	4
$(AC_{16})_5$ (**4**)	-CH_3 (L-Ala)	*n*-$C_{15}H_{31}$	5
$(DC_{16})_4$ (**5**)	-CH_2COO^- (L-Asp)	*n*-$C_{15}H_{31}$	4
$(KC_{18})_4$ (**6**)	-$(CH_2)_4NH_3^+$ (L-Lys)	*n*-$C_{17}H_{35}$	4
$(SC_{18})_4$ (**7**)	-CH_2OH (L-Ser)	*n*-$C_{17}H_{35}$	4

Figure 1. Cyclic peptides (**1**−**7**). Abbreviations: A, D, K, and S for L-alanine, L-aspartate, L-lysine, and L-serine, and C_{10}, C_{16}, and C_{18} for decanoyl, hexadecanoyl, and octadecanoyl side chains, respectively.

peptides by fixing the position and/or orientation of the side chain of the amino acid residues, both of which are known to be important factors determining the function. By using this strategy we have designed cyclic peptides, which are composed of an alternating natural amino acid/3-aminobenzoic acid sequence, and have shown indeed that these cyclic peptides exhibit the expected functions, such as strong binding of phosphates[65] and ester hydrolytic activity.[66] We have developed the molecular design of the cyclic peptide for an artificial ion channel and have actually synthesized cyclic peptides containing an alternating natural and unnatural amino acid sequence, the latter of which is 5-(*N*-alkanoylamino)-3-aminobenzoic acid with a long alkyl chain for feasible incorporation of the peptides into bilayer membranes (Figure 1).[67,68] In this design, a variety of natural amino acids can be introduced into the cyclic peptides, and the number of the component amino acids and the length of alkanoyl chains are readily altered. These cyclic peptides could be compared with the cyclic resorcinol tetramer bearing long alkyl chains,[62] which is more difficult to change the ring size due to some synthetic reasons. By investigating the ion channel properties of such cyclic peptides, we first examine whether the hydrophobic long alkanoyl (alkyl) chains can form a pore which acts as an ion channel. Moreover, we expect that such a simple ion channel should provide valuable information to elucidate the structure−function relationship of natural ion channels and to develop molecular devices which can convert chemical signals into electrical ones.

In this study, we have synthesized a series of symmetrical cyclic peptides with different ring size and alkanoyl chain, where each peptide contains a single kind of natural and unnatural amino acid. Here, the abbreviation for the cyclic peptides is given as a combination of the natural amino acid used, the length of alkanoyl chain, and the number (*n*) of the dipeptide units in peptides: e.g., $(AC_{10})_3$ corresponds to the cyclic peptide having L-alanine, *N*-decanoyl group (C_{10}), and three dipeptide units ($n = 3$). We report herein the synthesis of the cyclic peptides with a structural variety and their ion channel properties evaluated from the single channel study.

Scheme 1

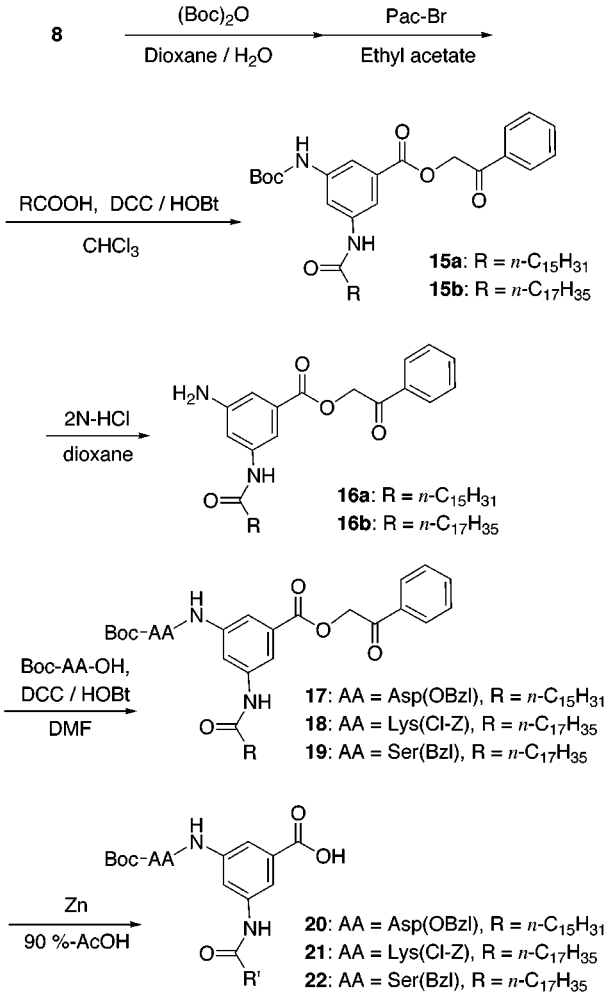


Results and Discussion

Synthesis and Structure of Cyclic Peptides. We synthesized cyclic peptides (**1**−**7**) containing an unnatural amino acid, 5-(*N*-alkanoylamino)-3-aminobenzoic acid, by coupling the precursor dipeptides prepared separately, because the unnatural amino acid is readily oxidized when the amino group is deprotected. For the amino acids which do not require protection of the side chain (in this work, L-alanine), ethyl 5-(*N*-alkanoylamino)-3-aminobenzoate was used as the protecting precursor (Scheme 1). In this synthesis, after 3,5-diaminobenzoic acid (**8**) was esterified to produce the ethyl ester (**9**), one of the two amino groups was protected with *tert*-butyloxycarbonyl (Boc) group to yield **10**. A C_{10} or C_{16} alkanoyl group was attached to the remaining amino group to give the corresponding compound **11a** or **11b**, respectively. Then, the Boc group was removed from **11a**,**b**, and the resulting 5-(*N*-alkanoylamino)-3-aminobenzoic acid ethyl ester was combined with a Boc-L-alanine. Finally, the ethyl ester protective group was removed with NaOH in aqueous ethanol to give the dipeptides (**14**), which are used in the solid-phase synthesis.

On the other hand, the peptides containing such amino acids that require the side chain protection (e.g., L-aspartate, L-lysine, and L-serine) were synthesized as

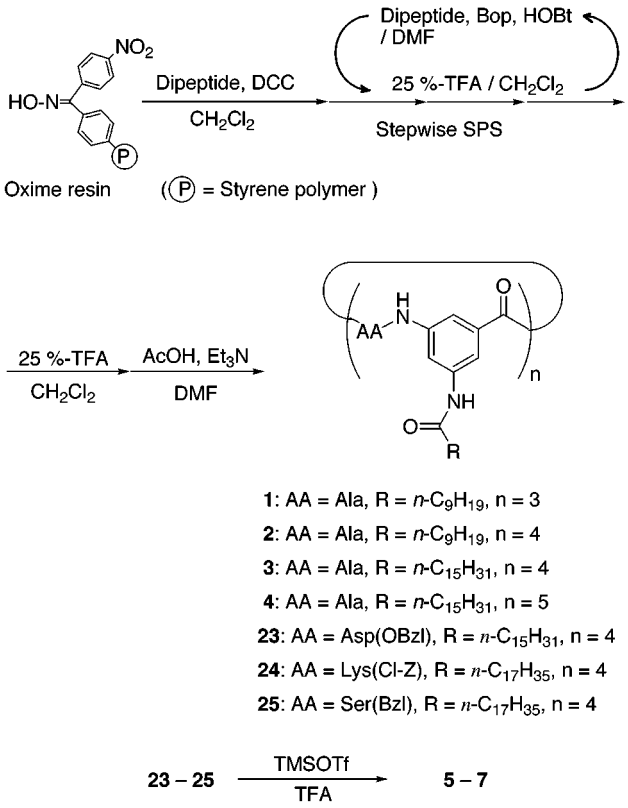
Scheme 2



shown in Scheme 2. In this synthesis, the phenacyl (Pac) group, which can be removed by reduction with zinc powder, was used for the protection of the benzoic acid to avoid the possible side reaction of the peptides upon removal of the protecting group. One of the amino groups of the unnatural amino acid was protected with Boc, the carboxylate with Pac by reacting with phenacyl bromide, and then the remaining free amino group was acylated by the DCC (*N*,*N*-dicyclohexylcarbodiimide)/HOBt (1-hydroxybenzotriazole) method. The Boc group of the compounds (**15**) was detached by treating with HCl in dioxane to produce the compounds (**16**) which were hardly crystallized, whereas the corresponding ethyl esters (**12**), which readily crystallize, were more easily purified and better handled. The compounds (**16**) were reacted with the amino acids with the protected side chain to yield dipeptides (**17**−**19**), followed by detaching the phenacyl group so as to be applicable to the solid-phase synthesis.

These dipeptides (**14**, **20**−**22**) were coupled and cyclized on an oxime resin to produce the cyclic peptides as shown in Scheme 3.[69−71] The cyclic peptides (**23**−**25**) containing L-aspartate, L-lysine, and L-serine, which further require removal of the protecting groups of the side chains, were

Scheme 3



therefore allowed to react with trimethylsilyl trifluoromethanesulfonate (TMSOTf) in trifluoroacetic acid (TFA) at 0 °C for 1 h.[72] All peptides synthesized in this work were identified by means of NMR and MS spectroscopy.

^{1}H NMR spectra of all cyclic peptides showed one equivalent signal for each proton in the component natural amino acid and 5-(*N*-alkanoyl)-3-aminobenzoic acid moieties, indicating that all cyclic peptides have symmetrical structures. Moreover, the ^{1}H NMR of $(AC_{10})_3$ and $(AC_{10})_4$ were almost independent of temperature from 25 through 80 °C in DMSO-d_6, although the amide protons showed downfield shifts of 0.3−0.4 ppm, probably as a result of aggregation through the hydrogen bonding interaction with water molecules present in the NMR solvent (see the Supporting Information). These results obtained from the NMR spectral study evidently indicate that all of the cyclic peptides are symmetrical and the conformations are thermodynamically stable. The ^{1}H NMR of $(AC_{16})_5$ showed broad peaks at 25 °C and sharp signals at higher temperatures, suggesting that the conformation is slightly flexible, although the peptide keeps the symmetrical structure at room temperature. These structural features are consistent with the molecular modeling structures of $(AC_{10})_3$, $(AC_{10})_4$, and $(AC_{16})_5$ which are obtained by the Insight/Discover program (from MSI, San Diego, CA). Figure 2 shows the top views of $(AC_{10})_3$, $(AC_{10})_4$, and $(AC_{16})_5$ exhibiting a triangular, square, and pentagonal shape with C3, C4, and C5 symmetry, respectively. Each cyclic peptide has a cavity at the center due to the rigidity of the unnatural amino acid component. Since these peptides have a common

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Table 1. Conductance Values of the Channels in a Symmetrical Solution of 500 mM KCl

channel	$(AC_{10})_3$	$(AC_{10})_4$	$(AC_{16})_4$	$(AC_{16})_5$	$(DC_{16})_4$	$(KC_{18})_4$	$(SC_{18})_4$
γ (pS) ± SD[a]	9.0 ± 1.4	8.0 ± 0.8	8.8 ± 1.4	9.1 ± 1.7	10.3 ± 1.8	8.6 ± 0.9	8.7 ±0.9
n[b]	14	8	22	9	14	6	4

[a] SD means standard deviation. [b] Numbers of the experiments.

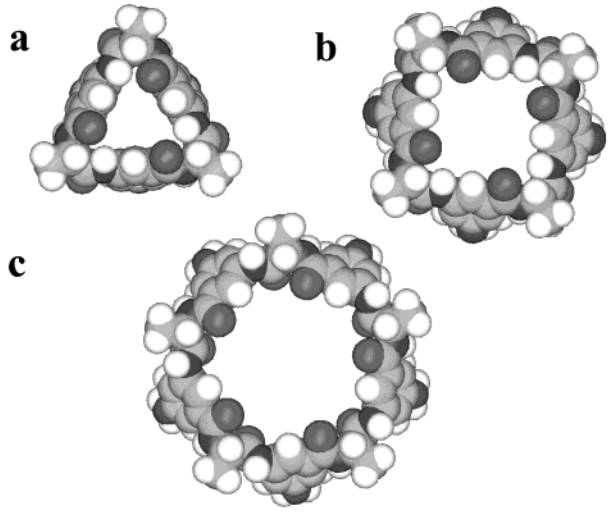

Figure 2. Top view of CPK models of the peptides composed of (a) 3, (b) 4, and (c) 5 dipeptide units. The alkanoyl chains are not shown for simplicity.

distance of 9.6 Å between the α-carbons of adjacent L-alanines located at the corners, the diameters of their cavities are ca. 4, 6, 11 Å for $(AC_{10})_3$, $(AC_{10})_4$, and $(AC_{16})_5$. The alkanoyl chains are aligned parallel in the same direction so that they can penetrate into bilayer membranes.

Single Ion Channel Properties of Cyclic Peptides. We have already reported that the cyclic octa-peptide $(AC_{16})_4$ forms an ion channel.[68] The detailed studies on the ion channel properties have revealed that the $(AC_{16})_4$ channel has the single-channel conductance of ca. 9 pS in a symmetrical 500 mM KCl solution and favors permeation of cations over anions with a permeability ratio (P_{Cl^-}/P_{K^+}) of 0.15. The channel is also K^+ selective over Na^+ cation with a permeability ratio (P_{K^+}/P_{Na^+}) of ca. l2.5, and furthermore the single-channel current is blocked by Ca^{2+}. From all available structural information, we have inferred that the $(AC_{16})_4$ channel is formed upon the tail-to-tail association of two $(AC_{16})_4$ molecules incorporated in bilayer membranes. We describe here the ion channel properties of the newly synthesized cyclic peptides as well as $(AC_{16})_4$.

Single Channel Conductance. The single channel conductance of individual cyclic peptides was examined in a symmetrical aqueous solution of KCl (500 mM) under the voltage clamp condition. All the cyclic peptides exhibited typical single channel characteristics, i.e., rapid transitions between the open and closed states, and stable current levels, when incorporated in lipid bilayer membranes (Figure 3). The latter behavior indicates that the structure of the open channel in the bilayer, once established, is very stable. The current−voltage ($I-V$) relationships of all the channels were linear under a symmetrical ionic condition (a typical result for $(DC_{16})_4$ in Figure 4). The single channel conductance was determined from the slope of $I-V$ curve obtained with a symmetrical 500 mM KCl solution. The results are

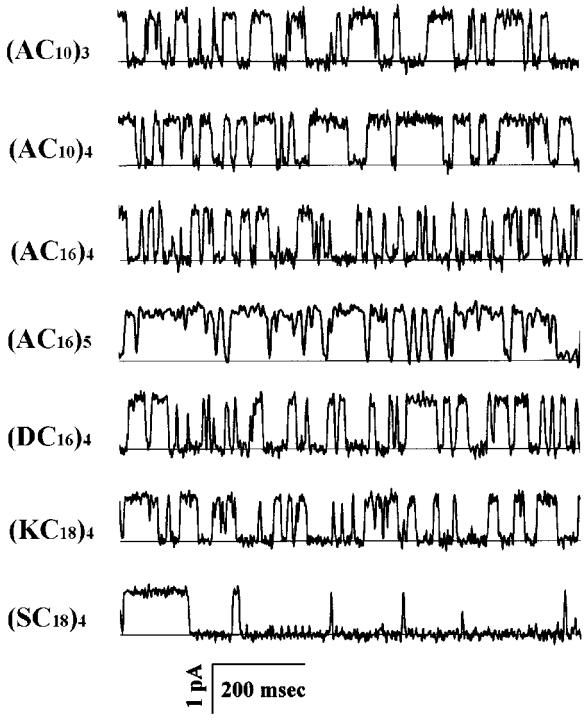

Figure 3. Representative single channel current traces observed for the membranes containing the cyclic peptides at +100 mV in a symmetric solution of 500 mM KCl.

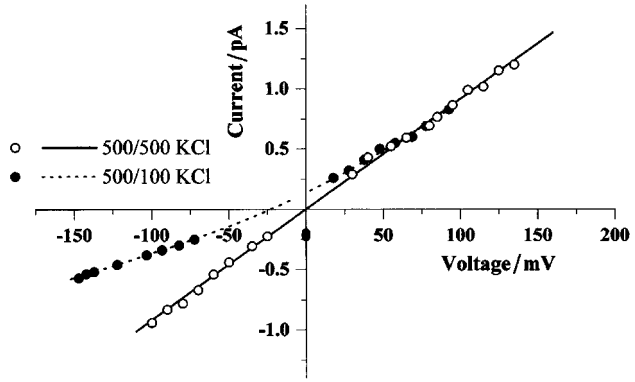

Figure 4. Current-voltage relationship of the $(DC_{16})_4$ channel in symmetrical (*cis*/*trans*, 500/500 mM KCl) and asymmetrical solutions (*cis*/*trans*, 500/100 mM KCl). Curves fitted to the data are drawn according to the GHK current equation.[1]

summarized in Table 1 with the number (n) of reported runs. No significant difference in the single channel conductance is seen despite the diversities in the alkanoyl chain length (10, 16, and 18), the number of constituted amino acids (6, 8, 10), and even the polarity of the peptides (containing neutral L-alanine ($(AC_{16})_4$) and L-serine ($(SC_{18})_4$), anionic L-aspartate ($(DC_{16})_4$), and cationic L-lysine ($(KC_{18})_4$)). It should be noted that these values are consistent with each other at 8.9 ± 0.7 pS, which is analogous to that (6.1 ± 0.8 pS) reported for the ion channel formed by the resorcinol tetramer, which also bears long C_{17} alkyl chains.[62]

Table 2. Charge Selectivity (Cl^-/K^+) of the Channels in Cis/Trans 500/100 mM KCl

channel	$(AC_{10})_3$	$(AC_{10})_4$	$(AC_{16})_4$[a]	$(AC_{16})_5$	$(DC_{16})_4$	$(KC_{18})_4$	$(SC_{18})_4$
V_r [mV] ± SD	−23.1 ± 1.9	−24.4 ± 3.2	−25.4 ± 1.1	−23.7 ± 1.7	−25.3 ± 2.2	−25.8 ± 3.1	−23.4 ± 2.6
n	4	5	3	4	3	3	3
P_{Cl^-}/P_{K^+}	0.19	0.17	0.15	0.18	0.15	0.14	0.19

[a] Reference 68.

Charge Selectivity. To determine whether the channels favor the permeation of cation or anion, similar experiment was performed under asymmetric ionic conditions (e.g. cis/trans 500/100 mM KCl), from which a curved $I-V$ relationship was obtained (see Figure 4, the broken line). This curvature is due to the charge selectivity of the channels and the concentration gradient of permeant ion. If a channel favors the permeation of K^+ over Cl^-, the cation, rather than the anion, moves from the cis to the trans side under the asymmetric condition. Such movement gives an excess positive charge in the trans side. As the trans side is usually defined as 0 mV, such ion movement builds up a negative electrical potential difference across the membrane. Under the voltage-clamp condition, the flow of current will reverse its direction at this potential (thus reversal potential). At the same time, currents (K^+) flow from cis to trans would be larger than that from trans to the cis, giving a super linear curve crossing at a negative voltage. Similarly, if a channel favors the permeation of Cl^- over K^+, we should observe an $I-V$ curve with a positive reversal potential. Therefore, by measuring the reversal potential, we can determine the charge selectivity of the channel and furthermore the permeability ratio, P_{Cl^-}/P_{K^+}, with the Goldman−Hodgkin−Katz (GHK) equation.[1] The results are listed in Table 2.

The $(AC_{16})_4$ channel has been already reported to favor permeation of K^+ over Cl^- with the permeation ratio P_{Cl^-}/P_{K^+} of 0.15.[68] The other cyclic peptides consisting of L-alanine, $(AC_{10})_3$, $(AC_{10})_4$, and $(AC_{16})_5$ showed similar permeation ratios, 0.17−0.19. This is probably due to the partially negatively charged carbonyl oxygens on the ring, which are situated at the entrance of the channel to facilitate permeation of K^+ rather than Cl^-.[68] This hypothesis would lead to an idea that the charge selectivity can be altered by changing the natural amino acids from L-alanine to charged ones such as L-aspartate (negative) and L-lysine (positive). However, the Cl^-/K^+ selectivity obtained for the $(DC_{16})_4$ and $(KC_{18})_4$ channels were 0.15 and 0.14, which are comparable to that obtained for the channels containing L-alanine. Moreover, $(SC_{18})_4$, which is neutral and probably more hydrophilic than $(AC_{16})_4$, has a similar Cl^-/K^+ selectivity as that of $(AC_{16})_4$. No significant difference was observed among the different channels despite their varied polarity of the side chain (alanine (or serine), aspartate, and lysine), ring size (3, 4, and 5 dipeptides), and chain lengths (10, 16, and 18 carbon atoms). It is noteworthy that the permeability ratios (0.14−0.19) we observed are slightly larger (less selective) than that (0.05) of the channel formed by the resorcinol tetramer with four C_{17} alkyl chains.[62] The results imply that the charge selectivity is determined by the structure of the cyclic headgroup. A possible reason of why the variation within the cyclic peptides scarcely affects the permeation ratio is discussed in the section of "Blockade by Ca^{2+}".

Cation Selectivity. We examined the monovalent cation selectivity between K^+ and Na^+ of the channels, which were limited to the L-alanine and L-aspartate peptides. The monovalent cation selectivity was examined under biionic conditions, which were established by adding the same amounts of Na^+ and K^+ gluconate solutions to the trans and cis side of the membrane, respectively, where gluconate anion is assumed not to permeate through the membrane. If the channel favors permeation of K^+ over Na^+, the negative reversal potential will be observed. As can be seen from Figure 5 (solid line), the $(DC_{16})_4$ channel shows a reversal potential of −21.7 mV under a biionic condition of cis/trans 500/500 mM $[K^+]/[Na^+]$. The permeability ratio P_{Na^+}/P_{K^+}, calculated from the reversal potential, is 0.40, indicating that the channel is more permeable for K^+ than for Na^+. In contrast, Li^+ could not permeate through the channel (for $(DC_{16})_4$, see the broken line in Figure 5) even though its ionic diameter (1.56 Å (Goldschmidt)) is much smaller than that of K^+ (2.66 Å).[73] This is probably due to difficulty of stripping some of water molecules surrounding Li^+, which is a necessary step for ion permeation through channels.[1] A similar tendency has been observed for the peptide channels constituted of L-alanine.[68] As summarized in Table 3, the permeability ratios, P_{Na^+}/P_{K^+}, among the channels are almost similar regardless of varying chain length (compare $(AC_{10})_4$ with $(AC_{16})_4$), ring size (compare $(AC_{16})_4$ with $(AC_{16})_5$), or charges (compare $(AC_{16})_4$ with $(DC_{16})_4$).

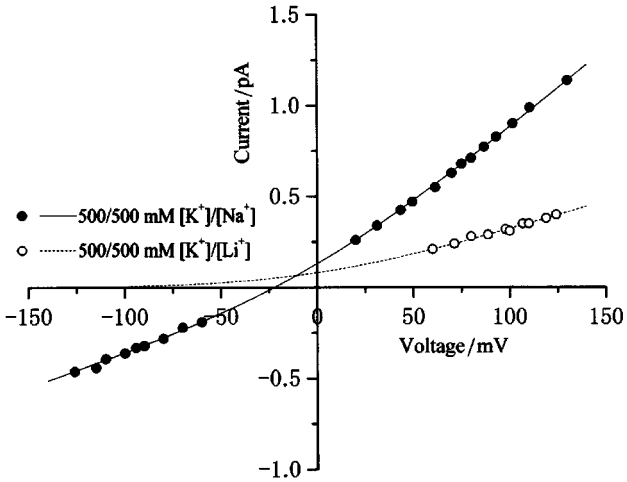

Figure 5. Current−voltage relationships of the $(DC_{16})_4$ channel in biionic solutions. Curves fitted to the data are drawn according to the GHK current equation.[1]

Table 3. Cation Selectivity (P_{Na^+}/P_{K^+}) of the Channels under Biionic Condition of 500/500 (K^+/Na^+ cis/trans mM)

channel	$(AC_{10})_4$	$(AC_{16})_4$[a]	$(AC_{16})_5$	$(DC_{16})_4$
V_r [mV] ± SD	−21.2 ± 2.8	−22.1 ± 0.8	−23.6 ± 1.9	−21.7 ± 3.3
n	4	3	3	3
P_{Na^+}/P_{K^+}	0.41	0.39	0.37	0.40

[a] Reference 68.

(73) Sinha, A. P. *Struct. Bonding* **1976**, *25*, 69.

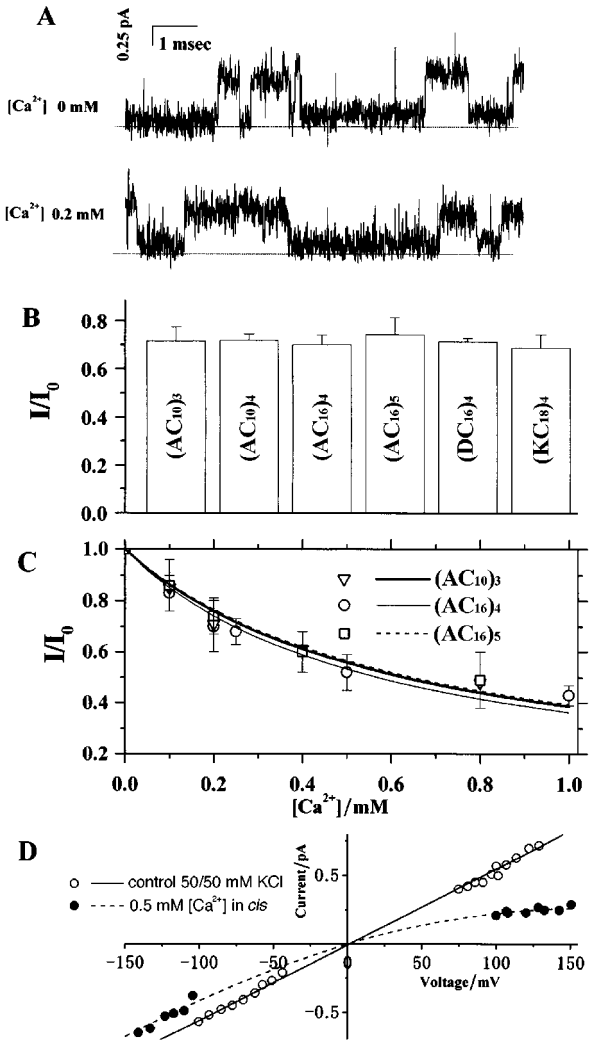

Figure 6. Dose and voltage dependent blockade of single channel currents by Ca^{2+}. (A) Single channel current traces for the $(AC_{16})_4$ channel at +100 mV in the presence of 0 and 0.2 mM Ca^{2+}. (B) Relative single channel currents (I/I_0) in the presence of 0.2 mM Ca^{2+}. I and I_0 are the currents at +100 mV in the presence and absence of Ca^{2+}. Curves fitted to the data are drown according to eq 1. (C) Dose-dependent blockade of single channel currents by Ca^{2+} at +100 mV. (D) Voltage-dependent blockade of single channel current: current−voltage relationships of the $(DC_{16})_4$ channel before and after adding 0.5 mM Ca^{2+} into the cis side of the solution.

Blockade by Ca^{2+}. Addition of 0.2 mM Ca^{2+} to one side of the chamber containing a KCl (500 mM) solution decreased the single channel currents of the $(AC_{16})_4$ channel (Figure 6A). This result indicates that Ca^{2+} blocks the ion channel. The relative currents (I/I_0) in the presence and absence of 0.2 mM Ca^{2+} are almost similar for all the channels tested despite their different structures (Figure 6B). This suggests that Ca^{2+} blocks these cyclic peptides in a similar manner. Furthermore, relative single-channel currents (I/I_0) could be fitted with a single site titration curve according to

$$I/I_0 = \{(1 + [Ca^{2+}]/K_d(V))\}^{-1} \quad (1)$$

where K_d (V) is the dissociation constant at a given voltage V, $[Ca^{2+}]$ is the concentration of Ca^{2+} in the solution, I and I_0 are the current at given voltage with or without Ca^{2+}. This result suggests that Ca^{2+} blocks the single channel currents in a one to one fashion. From these titration curves, the dissociation constant of Ca^{2+} at +100 mV was calculated to be ca. 0.6 mM throughout the channel tested ($(AC_{16})_4$, $(AC_{10})_3$, and $(AC_{16})_5$) despite their different pore sizes.

Table 4. Blocking Parameters of Ca^{2+} on the Channels

peptide	$(AC_{16})_4$[a]	$(AC_{16})_5$	$(DC_{16})_4$
$z\delta \pm$ SD	0.07 ± 0.03	0.12 ± 0.04	0.11 ± 0.03
$K_d(0)$ [mM] ± SD	0.81 ± 0.11	0.78 ± 0.14	0.87 ± 0.16

[a] Reference 68.

The blocking effect of Ca^{2+} was voltage dependent. The single channel current for the peptide channels was reduced more effectively at positive voltages than at negative ones in the presence of 0.5 mM Ca^{2+} in the cis side. As a typical example, the $I-V$ relationships for the $(DC_{16})_4$ channel in the presence and absence of 0.5 mM Ca^{2+} in the cis side are shown in Figure 6D. For the voltage-dependent blockade by Ca^{2+}, the relative conductance value (γ/γ_0) is defined as:

$$\gamma/\gamma_0 = \{1 + [Ca^{2+}]/K_d(0)\exp(z\delta FV/RT)\}^{-1} \quad (2)$$

according to the Woodhull's blocking theory,[74] where $K_d(0)$ is an apparent zero voltage dissociation constant for Ca^{2+} and $z\delta$ is a fractional electrical distance between the binding site and the cis entrance of the channel. Thus, analysis of the $I-V$ data in the presence and absence of Ca^{2+} gives the dissociation constant at 0 mV ($K_d(0)$) and the electric distance ($z\delta$). These values for the $(DC_{16})_4$ channel are summarized in Table 4 along with those for $(AC_{16})_4$ and $(AC_{16})_5$. The $K_d(0)$ values are kept constant at ca. 0.8−0.9 mM regardless of their different structures. The electric distances ($z\delta$) are also similar (0.07−0.12) for these three cyclic peptides. The $z\delta$ values are quite small, indicating that the Ca^{2+} binding site is located very close to the channel entrance. It should be noted that Ca^{2+} can block the channels from either side of the membrane in the same manner, suggesting that these channels have two equivalent binding sites at the both sides of the membrane and therefore the symmetrical structure throughout the bilayer membrane. Taking into account the highly hydrophilic nature of Ca^{2+}, the most hydrophilic cyclic peptide moiety is thought to be the binding site for Ca^{2+}. Therefore, the small $z\delta$ values suggest that the peptide ring should be situated at the entrance of the channel. As electrophysiological properties of all the channels are similar, we may speculate that all the channels take similar structure in the bilayer membrane as that of the $(AC_{16})_4$ channel.[68] That is, each channel is formed from a dimer of the cyclic peptide, the peptide ring of which forms the channel entrance while its alkanoyl chains line across the membrane to form the channel pore.

Despite the different structural features between each of the channels, all the single channel properties were similar. The reason for this discrepancy is not exactly known, but might be explained by the following considerations. The fact that the charge selectivity and the Ca^{2+}-blocking behavior are scarcely affected by the difference in side chain charge of the cyclic peptides may be accounted for if the charged residues are located far from the channel entrance. Actually, the molecular

(74) Woodhull, A. M. *J. Gen. Physiol.* **1973**, *61*, 687.

modelings by the InsightII/Discover program indicate that the distance between the carboxyl oxygen in Asp and the peptide ring is over 6 Å and that between the nitrogen atom in Lys and the ring is over 9 Å. According to the Gouy−Chapman's surface potential theory, the Debye length, which is a distance to which the electrostatic effect of a surface charge can reach in solution, is only 4.6 Å in seawater.[1] Therefore, charged atoms of the side chains are too far to significantly affect the ion permeation.

It is expected that the larger the size of the peptide ring, the easier the ions to enter into the pore of the channel, and consequently the larger the conductance of the channel. But no significant difference of the conductance between different peptides with ring size ranging from 3 to 5 was observed. This might be explained by a consideration that the enlargement will weaken the interaction between the ion and the peptide ring at the same time. Based on the proposed structures of the channels, the interaction between the ion and channel during the ion permeation can be decomposed into the interaction between the ion and the peptide ring, and the interaction between the ion and the alkanoyl chains. Obviously, the only common part of the interaction between the ion and all of the peptide is the alkanoyl chains. Therefore, the small influence of the structural variation on the single channel conductance suggests that the process of ion passing through the hydrophobic alkanoyl chain region, is the rate-limiting step for ions permeation through all of these channels. This inference is consistent with energy profiles for ions permeation through the $(AC_{16})_4$ channel, which was calculated based on a three-energy-barrier two-binding-site (3B2S) model of Eyring rate theory.[68] It is well-known that the current of an ion permeating across a channel is an integration through the whole energy profile the ion felt during its permeation.[75] Therefore, the alkanoyl chain region, which is much longer than the peptide ring region, contributes significantly to the current, therefore the conductance, of the peptide channels. The length of the alkanoyl chain in the channels did not influence the conductance, although one would expect a lower conductance level for a longer pore. Such apparently unexpected behavior would be rationalized by assuming that the degree of interdigitation of the alkanoyl chains increases with increasing chain length to afford a more ordered pore, which facilitates the ion permeation eventually giving analogous conductances for a series of peptides with different alkanoyl chains.

Conclusion

We have designed and synthesized artificial ion channels based on cyclic peptides which consist of alternating natural and unnatural amino acids. The unnatural amino acid, 5-(*N*-alkanoylamino)-3-aminobenzoic acid, renders the cyclic peptides structurally rigid, endowing an inherent pore through which ions can permeate. As has been demonstrated for the $(AC_{16})_4$ channel,[68] the cyclic peptide builds up a single ion channel by forming a tail-to-tail associated dimer so as to align the hydrophobic alkanoyl chains in bilayer membranes. In this work, the cyclic peptides with structural varieties, including the alkanoyl chain length, the ring size of cyclic peptide, and the type of natural amino acid, have been synthesized. All the peptides were demonstrated to form ion channels by the single-channel recording technique. The single channel properties of the cyclic peptides were investigated in terms of single channel conductance, charge selectivity, and cation selectivity. These properties were almost similar among the cyclic peptide channels despite the divergent structural variations. The channels were blocked by Ca^{2+} in a voltage-dependent manner. The blocking experiments revealed that Ca^{2+} block the ion channels from both sides, clearly indicating that the cyclic peptide aggregates to give a symmetrical channel. The parameter of $z\delta$ is quite small, suggesting that the binding site is very close to the entrance of the channel. These results are consistent with an idea that each channel is formed from a dimer of the peptide, the ring of which forms the channel entrance while its alkanoyl chains line the channel pore. On the basis of this structure, we concluded that the rate-limiting step of ion conduction occurs at the hydrophobic alkanoyl chain region.[76] Since the hydrophobic region exists widely in natural channel proteins, this work provided a good model to study the mechanism of ion permeation through a hydrophobic region of natural channels.

Experimental Section

General. The Boc-L-amino acids were purchased from Watanabe Chemical Industries, Ltd., except for Boc-L-Ala-OH which was prepared in our laboratory. The other chemicals of reagent grade were purchased from Tokyo Kasei Kogyo Co., Ltd. and Nacalai Tesque, Inc. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 at 400 MHz with TMS as an internal standard. Structural models of the channels were constructed on a Silicon Graphics Indigo^2 workstation by using Insight II 95 and Discover 95 (MSI, San Diego, CA) programs as previously described.[77]

Syntheses of Cyclic Peptides

3,5-Diaminobenzoic Acid Ethyl Ester (9). Thionyl chloride (10 cm^3) was added dropwise to dry ethanol (30 cm^3) in an ice bath. 3,5-Diaminobenzoic acid (**8**, 5 g) was added to the solution, which was further refluxed for 1 day. After evaporation, the resulting solid was dissolved in ethyl acetate, and the solution was washed with 4% aqueous solution of sodium hydrogen carbonate ($NaHCO_3$) (10 cm^3 × 3) and then with water to remove the remaining starting material **8**. The ethyl acetate solution was dried over anhydrous $MgSO_4$ and then evaporated to yield the product **9** as a solid: 5.5 g, 93% yield. ^1H NMR ($CDCl_3$) δ 6.79 (2H, s), 6.18 (1H, s), 4.44 (2H, q), 3.13 (4H, br), 1.36 (3H, t). Anal. Calcd for $C_9H_{12}O_2N_2$: C, 59.99; H, 6.71; N, 15.55. Found: C, 60.02; H, 6.60; N, 15.47.

3-(*N*-*tert*-Butoxycarbonylamino)-5-aminobenzoic Acid Ethyl Ester (10). To 60 cm^3 of dioxane−water (2:1 v/v) solution were added **9** (2 g), triethylamine (Et_3N, 3.3 cm^3), and di-*tert*-butyl dicarbonate ($(Boc)_2O$, 2.1 g). The solution was gently stirred at 0 °C for 1 h and at room temperature for 10 h, and then the solvent was evaporated. The oil residue was dissolved in ethyl acetate (50 cm^3), and the solution was

(75) Levitt, D. G. *Annu. Rev. Biophys. Chem.* **1986**, *15*, 29.

(76) An alternative channel structure model would involve a pore formed by aggregation of the cyclic peptides. Although the present results cannot rigorously exclude such a possibility, this model is not compatible with the fact that the ion channel properties, such as gating kinetics, are not appreciably affected by the charge of the cyclic peptides; charged peptides are more difficult to aggregate with each other than neutral ones. This model can reasonably account for the observed channel properties independent of the charge, as the rate-limiting step is the ion permeation through the hydrophobic alkanoyl chain region.

(77) Qi, Z.; Sokabe, M. *Biophys. Chem.* **1998**, *71*, 35.

washed with 10% aqueous citric acid solution (20 $cm^3 \times 3$) and with water. The ethyl acetate solution was dried over $MgSO_4$ and evaporated to give the product **10**: 1.8 g, 83% yield. ^1H NMR ($CDCl_3$) δ 7.26 (1H, s), 7.13 (1H, s), 7.02 (1H, s), 6.55 (1H, br), 4.45 (2H, q), 1.51 (9H, s), 1.36 (3H, t). Anal. Calcd for $C_{14}H_{20}O_4N_2$: C, 59.99; H, 7.19; N, 9.99. Found: C, 59.99; H, 7.29; N, 9.83.

3-(*N*-*tert*-Butoxycarbonylamino)-5-(*N*′-decanoylamino)benzoic Acid Ethyl Ester (11a). Triethylamine (3 cm^3) and decanoic anhydride (4 g) were added to **10** (5 g) in chloroform (50 cm^3). The mixture was stirred at room temperature for 1 day, and then the solvent was evaporated. The oily residue was dissolved in ether−petroleum ether, and the solution was allowed to stand at room temperature for 3 h to give the product **11a** as crystals: 5.8 g, 75% yield. ^1H NMR ($CDCl_3$) δ 8.05 (1H, s), 7.83 (1H, s), 7.77 (1H, s), 7.66 (1H, s), 6.95 (1H, s), 4.38 (2H, q), 2.36 (2H, t), 1.72 (2H, m), 1.51 (9H, s), 1.38 (3H, t), 1.26 (12H, br), 0.89 (3H, t); ^{13}C NMR ($CDCl_3$) δ 169.3, 166.2, 152.7, 139.3, 138.8, 131.7, 115.0 (2C), 114.0, 81.0, 61.2, 37.7, 33.8, 29.4 (3C), 28.3 (3C), 25.5, 22.6 (2C), 14.3 (2C). Anal. Calcd for $C_{24}H_{38}O_5N_2$: C, 66.33; H, 8.81; N, 6.45. Found: C, 66.35; H, 8.77; N, 6.47.

3-(*N*-*tert*-Butoxycarbonylamino)-5-(*N*′-hexadecanoylamino)benzoic Acid Ethyl Ester (11b). Triethylamine (5.6 cm^3) and dicyclohexylcarbodiimide (DCC, 10 g) were added to chloroform (60 cm^3) containing **10** (9.3 g), hexadecanoic acid (10 g), and 1-hydroxybenzotriazole (HOBt, 6.2 g) at 0 °C. The mixture was stirred continuously at 0 °C for 1 h and then at room temperature for 20 h. The solution was evaporated to yield an oily residue, which was dissolved in ethyl acetate (60 cm^3) and 10% aqueous citric acid solution (30 cm^3). The solution was stirred for 30 min to generate *N*,*N*′-dicyclohexylurea (DCUrea), which was filtered off. The ethyl acetate phase was washed successively with 10% aqueous citric acid solution (30 $cm^3 \times 3$), water, 4% aqueous $NaHCO_3$ solution (30 $cm^3 \times 3$), and then water (30 $cm^3 \times 1$). The ethyl acetate solution was dried over $MgSO_4$ and was evaporated. The resultant was dissolved in ether−petroleum ether, and the solution was allowed to stand at room temperature for 5 h to produce the product **11b** as crystals: 16 g, 93% yield. ^1H NMR ($CDCl_3$) δ 8.11 (1H, s), 7.83 (1H, s), 7.77 (1H, s), 7.42 (1H, s), 6.78 (1H, s), 4.38 (2H, q), 2.38 (2H, t), 1.74 (2H, m), 1.50 (9H, s), 1.36 (3H, t), 1.26 (24H, br), 0.91 (3H, t); ^{13}C NMR ($CDCl_3$) δ 171.6, 166.1, 152.6, 139.3, 138.8, 131.8, 115.0 (2C), 113.8, 81.0, 61.2, 37.8, 31.9, 29.7 (9C), 28.3 (3C), 25.5, 22.7 (2C), 14.3 (2C). Anal. Calcd for $C_{30}H_{50}O_5N_2$: C, 69.46; H, 9.72; N, 5.40. Found: C, 69.44; H, 9.78; N, 5.28.

3-Amino-5-(*N*-decanoylamino)benzoic Acid Ethyl Ester (12a). To dry dioxane (35 cm^3) containing **11a** (5 g), 4 M HCl−dioxane (35 cm^3) was added, and the solution was allowed to stand at room temperature for 3 h to yield **12a** as crystals. By adding ether (100 cm^3) to the solution, the further precipitated crystals were collected by filtration: 3.4 g, 73% yield. ^1H NMR (DMSO-d_6) δ 7.29 (1H, s), 7.28 (1H, s), 7.21 (1H, s), 4.28 (2H, q), 2.27 (2H, t), 1.56 (2H, br), 1.31 (3H, t), 1.25 (12H, br), 0.87 (3H, t). Anal. Calcd for $C_{19}H_{30}O_3N_2$: C, 68.23; H, 9.04; N, 8.38. Found: C, 68.12; H, 9.13; N, 8.32.

3-Amino-5-(*N*-hexadecanoylamino)benzoic Acid Ethyl Ester (12b). Synthesis of **12b** was performed from the compound **11b** (16.0 g) according to the same procedures employed in the synthesis of **12a**: 13 g, 99% yield. ^1H NMR ($CDCl_3$) δ 7.57 (1H, s), 7.26 (1H, s), 7.18 (1H, s), 7.17 (1H, s), 4.36 (2H, q), 2.35 (2H, t), 1.72 (2H, br), 1.37 (3H, t), 1.25 (24H, br), 0.90 (3H, t); ^{13}C NMR ($CDCl_3$) δ 171.6, 166.4, 147.4 (2C), 139.1, 111.5, 110.5, 110.4, 61.0, 37.9, 31.9, 29.7 (6C), 25.6, 22.7 (2C), 14.3 (2C). Anal. Calcd for $C_{25}H_{42}O_3N_2$: C, 71.73; H, 10.11; N, 6.69. Found: C, 71.74; H, 10.24; N, 6.69.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-L-alanyl)amino]-5-(*N*′-decanoylamino)benzoic Acid Ethyl Ester (13a). Boc-Ala-OH (2.3 g) and **12a** (3.4 g) were dissolved in DMF (20 cm^3), to which HOBt (0.3 g), triethylamine (14.0 cm^3), and DCC (2.5 g) were added at 0 °C. The solution was stirred at 0 °C for 1 h and then at room temperature for additional 20 h. After evaporation, the oily residue was dissolved in ethyl acetate (30 cm^3) and 10% aqueous citric acid solution (20 cm^3). The solution was stirred for 30 min and was filtrated in order to remove DCUrea. The ethyl acetate phase was washed successively with 10% aqueous citric acid solution (20 $cm^3 \times 3$), water (20 cm^3), 4% $NaHCO_3$ aqueous solution (20 $cm^3 \times 3$), and water (20 cm^3). The ethyl acetate solution was dried over $MgSO_4$ and evaporated. The resulting oil was purified by silica gel chromatography (Wakogel C-200) with $CHCl_3$−CH_3OH (30:1 v/v). A mixture of ether−petroleum ether was added to the oily compound to produce a solid: 4.5 g, 88% yield. ^1H NMR (DMSO-d_6) δ 10.13 (1H, s), 10.09 (1H, s), 8.24 (1H, s), 7.93 (1H, s), 7.91 (1H, s), 7.07 (1H, d, J = 6.84 Hz, amide(Ala)), 4.32 (2H, q, CH_2(Et)), 4.10 (1H, m), 2.49 (2H, t), 1.57 (2H, br), 1.36 (9H, s), 1.32 (3H, t), 1.26 (3H, d, J = 6.84 Hz, β-CH_3-(Ala)), 1.21 (12H, br), 0.84 (3H, t). Anal. Calcd for $C_{27}H_{43}O_6N_3$: C, 64.13; H, 8.57; N, 8.31. Found: C, 64.08; H, 8.57; N, 8.29.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-L-alanyl)amino]-5-(*N*′-palmitoylamino)benzoic Acid Ethyl Ester (13b). The titled compound was synthesized from Boc-Ala-OH (4.5 g) and **12b** (5 g) according to the same procedures employed for **13a**: 4 g, 60% yield. ^1H NMR (DMSO-d_6) δ 10.13 (1H, s), 10.08 (1H, s), 8.24 (1H, s), 7.93 (2H, s), 7.08 (1H, d, J = 6.83 Hz, amide-(Ala)), 4.33 (2H, q), 4.12 (1H, m), 2.32 (2H, t), 1.60 (2H, br), 1.38 (9H, s), 1.33 (3H, t), 1.30 (3H, d), 1.22 (24H, br), 0.84 (3H, t); ^{13}C NMR (DMSO-d_6) δ 172.5, 171.6, 165.5, 139.9 (2C), 130.5, 114.4 (2C), 112.5, 78.0, 60.8, 50.2, 36.4, 31.3−28.2 (12C), 25.0, 22.1, 17.8, 14.2 (2C). Anal. Calcd for $C_{33}H_{55}O_6N_3$: C, 67.20; H, 9.40; N, 7.13. Found: C, 66.97; H, 9.39; N, 7.31.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-L-alanyl)amino]-5-(*N*′-decanoylamino)benzoic Acid (14a). To an ethanol solution (10 cm^3) of **13a** (6 g) was added 1 M NaOH aqueous solution (15 cm^3), and the resultant mixture was vigorously stirred at room temperature for 1 day. After evaporation, the residue was dissolved in ethyl acetate (30 cm^3), which was washed with 10% aqueous citric acid solution (30 $cm^3 \times 3$) and with water (30 $cm^3 \times 1$). The ethyl acetate phase was further dried over $MgSO_4$ and evaporated. A mixture of ether−petroleum ether was added to the resulting oil to produce a solid: 4.2 g, 73% yield. ^1H NMR (DMSO-d_6) δ 10.09 (1H, s), 10.05 (1H, s), 8.18 (1H, s), 7.91 (1H, s), 7.89 (1H, s), 7.09 (1H, d, J = 6.96 Hz, amide(Ala)), 4.11 (1H, m), 2.50 (2H, t), 1.60 (2H, br), 1.38 (9H, s), 1.25 (15H, br), 0.87 (3H, t). Anal. Calcd for $C_{25}H_{39}O_6N_3$: C, 62.86; H, 8.23; N, 8.80. Found: C, 62.51; H, 8.25; N, 8.70.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-L-alanyl)amino]-5-(*N*′-hexadecanoylamino)benzoic Acid (14b). The synthesis of **14b** was performed by using **13b** (4 g) according to the same procedures employed for **14a**: 3 g, 76% yield. ^1H NMR (DMSO-d_6) δ 10.08 (1H, s), 10.03 (1H, s), 8.18 (1H, s), 7.92 (2H, s), 7.06 (1H, d, J = 6.83 Hz, amide(Ala)), 4.12 (1H, m), 2.31 (2H, t), 1.58 (2H, br), 1.26 (9H, s), 1.23 (27H, d), 0.86 (3H, t). Anal. Calcd for $C_{31}H_{51}O_6N_3 \cdot 0.5H_2O$: C, 65.23; H, 9.18; N, 7.36. Found: C, 65.45; H, 8.29; N, 7.32.

3-(*N*-*tert*-Butoxycarbonylamino)-5-(*N*′-hexadecanoylamino)benzoic Acid Phenacyl Ester (15a). In the synthesis of **15a**, one amino group of **8** with *tert*-Boc was first protected by using $(Boc)_2O$ (21.5 g) and **8** (15 g) according to the similar procedures employed for **10** to produce 3-(*tert*-butoxycarbonylamino)-5-aminobenzoic acid: 20 g, 82% yield. Esterification of the carboxylic group of this compound with phenacyl group was performed by reacting the intermediate product (6.7 g) mentioned above with triethylamine (3.7 cm^3) and 2-bromoacetophenone (4.8 g) in ethyl acetate to produce 3-(*N*-*tert*-butoxycarbonylamino)-5-aminobenzoic acid phenacyl ester: 9.4 g, 95% yield. The title compound **15a** was synthesized by coupling of the phenacyl ester (6.9 g) with hexadecanoic acid (4.8 g) according to the same procedure employed for **11b**. The crude product **15a** was purified by using a column chromatography (Wakogel C-200) with $CHCl_3$−CH_3OH (30:1 v/v) eluent and was recrystallized from ether−petroleum ether: 8.3 g, 75% yield. ^1H NMR ($CDCl_3$) δ 8.00 (1H, br), 7.96 (3H, d), 7.81 (1H, s), 7.64 (1H, t), 7.51 (3H, d), 6.85 (1H, br), 5.55 (2H, s), 2.35 (2H, t), 1.69 (2H, br), 1.50 (9H, s), 1.25 (24H, br), 0.89 (3H, t). Anal. Calcd for $C_{36}H_{52}O_6N_2$: C, 71.02; H, 8.61; N, 4.60. Found: C, 68.21; H, 7.64; N, 5.15.

3-(*N*-*tert*-Butoxycarbonylamino)-5-(*N*′-octadecanoylamino)benzoic Acid Phenacyl Ester (15b). The compound **15b** was similarly synthesized according to the procedures employed for **15a** by using octadecanoic acid (6.8 g) and 3-(*tert*-butoxycarbonylamino)-5-aminobenzoic acid phenacyl ester (7.4 g) in the final step: 10 g, 80% yield. ^{1}H NMR ($CDCl_3$) δ 8.03 (1H, s), 7.96 (1H, s), 7.94 (2H, d), 7.83 (1H, s), 7.80 (2H, s), 7.59 (1H, t), 6.79 (1H, s), 5.54 (2H, s), 2.35 (2H, t), 1.61 (2H, br), 1.51 (9H, s), 1.25 (28H, br), 0.89 (3H, t). Anal. Calcd for $C_{38}H_{56}O_6N_2$: C, 71.67; H, 8.86; N, 4.40. Found: C, 72.67; H, 10.41; N, 3.94.

3-Amino-5-(*N*-hexadecanoylamino)benzoic Acid Phenacyl Ester (16a). Deprotection of the Boc group of **15a** was performed by adding 4 M HCl−dioxane (47 cm^3) to the dry dioxane solution (47 cm^3) of **15a** (9.7 g). The solution was allowed to stand at room temperature for 3 h and evaporated. The resulting solid was dissolved in ethyl acetate (60 cm^3), and the solution was washed with 10% aqueous citric acid solution (50 cm^3 × 4) and then water (50 cm^3 × 1). The ethyl acetate solution was dried with $MgSO_4$ and evaporated to yield **16a**: 7.4 g, 75% yield. ^{1}H NMR (DMSO-d_6) δ 10.42 (1H, s), 8.03 (3H, d), 7.95 (1H, s), 7.75 (1H, t), 7.61 (3H, d), 5.79 (2H, s), 2.37 (2H, t), 1.60 (2H, br), 1.25 (24H, br), 0.87 (3H, t). Anal. Calcd for $C_{31}H_{44}O_4N_2$·HCl: C, 68.30; H, 8.32; N, 5.14. Found: C, 64.96; H, 7.17; N, 6.07.

3-Amino-5-(*N*-stearoylamino)benzoic Acid Phenacyl Ester (16b). The synthesis of **16b** was performed from **15b** (6 g) according to the same procedures employed for **16a** (3.2 g, 59% yield). ^{1}H NMR (DMSO-d_6) δ 10.22 (1H, s), 8.02 (1H, d), 8.00 (1H, s), 7.91 (1H, s), 7.78 (1H, s), 7.73 (1H, t), 7.60 (2H, d), 5.76 (2H, s), 2.33 (2H, t), 1.58 (2H, br), 1.22 (28H, br), 0.86 (3H, t). Anal. Calcd for $C_{33}H_{48}O_4N_2$·HCl: C, 69.14; H, 8.62; N, 4.89. Found: C, 68.51; H, 8.42; N, 4.85.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-*O*-β-benzyl-L-aspartyl)-amino]-5-(*N*′-palmitoylamino)benzoic Acid Phenacyl Ester (17). The dipeptide **17** was synthesized from Boc-Asp(OBzl)-OH (2.1 g) and **16a** (3 g) by the same procedure as described for **13a**: 3.3 g, 67% yield. ^{1}H NMR (DMSO-d_6) δ 10.34 (1H, s), 10.15 (1H, s), 8.27 (2H, d), 8.03 (2H, d), 7.74 (1H, s), 7.61 (2H, t), 7.36 (5H, m), 7.30 (1H), 5.76 (2H, s), 5.11 (2H, s), 4.53 (1H, br), 2.89−2.68 (2H, m), 2.33 (2H, t), 1.59 (2H, br), 1.39 (9H, s), 1.23 (24H, br), 0.86 (3H, t). Anal. Calcd for $C_{47}H_{63}O_9N_3$: C, 69.34; H, 7.80; N, 5.16. Found: C, 68.95; H, 8.29; N, 5.52.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-*N*′-ϵ-2-chlorocarbobenzoxy-L-lysyl)amino]-5-(*N*′-octadecanoylamino)benzoic Acid Phenacyl Ester (18). The synthesis of **18** was similarly performed from Boc-Lys(Cl-Z)-OH (1.2 g) and **16b** (1.3 g): 1.8 g, 80% yield. ^{1}H NMR (DMSO-d_6) δ 10.20 (1H, s), 10.13 (1H, s), 8.27 (1H, s), 8.03 (2H, d), 8.01 (2H, d), 7.73 (1H, s), 7.61 (2H, t), 7.36 (2H, d), 7.34 (3H, d), 7.01 (1H, d, J = 7.32 Hz, amide(Lys)), 5.76 (2H, s), 5.07 (2H, s), 4.05 (1H, br), 3.01 (2H, br), 2.33 (2H, t), 1.60 (4H, br), 1.38 (11H, s), 1.22 (30H, br), 0.85 (3H, t). Anal. Calcd for $C_{52}H_{73}O_9N_4Cl$: C, 66.90; H, 7.88; N, 6.00. Found: C, 64.40; H, 7.52; N, 6.24.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-*O*-benzyl-L-seryl)amino]-5-(*N*′-octadecanoylamino)benzoic Acid Phenacyl Ester (19). The dipeptide **19** was similarly synthesized from Boc-Ser(Bzl)-OH (1.7 g) and **16b** (2.7 g): 2.5 g, 65% yield. ^{1}H NMR (DMSO-d_6) δ 10.32 (1H, s), 10.12 (1H, s), 8.28 (2H, d), 8.05 (2H, d), 7.72 (1H, s), 7.60 (2H, t), 7.32 (5H, m), 7.05 (1H, br), 5.74 (2H, s), 4.53 (2H, s), 4.43 (1H, br), 3.65 (2H, m), 2.33 (2H, t), 1.59 (2H, br), 1.39 (9H, s), 1.23 (28H, br), 0.86 (3H, t). Anal. Calcd for $C_{48}H_{67}O_8N_3$: C, 70.82; H, 8.30; N, 5.16. Found: C, 70.82; H, 8.38; N, 5.18.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-*O*-β-benzyl-L-aspartyl)-amino]-5-(*N*′-hexadecanoylamino)benzoic Acid (20). To the 90% acetic acid solution (15 cm^3) of **17** (3.3 g) was added zinc powder (8.0 g), and the mixture was vigorously stirred at room temperature for 1 day. To remove the unreacted zinc powder, the solution was filtrated and then evaporated. The resulting oil was dissolved in ethyl acetate (50 cm^3), which was washed with 10% aqueous citric acid solution (30 cm^3 × 4) and with water (30 cm^3 × 1). The ethyl acetate solution was dried over $MgSO_4$ and was evaporated. The crude compound was recrystallized with ether−petroleum ether: 2.1 g, 77% yield. ^{1}H NMR (DMSO-d_6) δ 10.23 (1H, s), 10.05 (1H, s), 8.19 (1H, s), 7.92 (2H, d), 7.35 (5H, m), 7.27 (1H), 5.11 (2H, s), 4.51 (1H, br), 2.87−2.69 (2H, m), 2.31 (2H, t), 1.58 (2H, br), 1.39 (9H, s), 1.23 (24H, br), 0.86 (3H, t). Anal. Calcd for $C_{39}H_{57}O_8N_3$: C, 67.31; H, 8.26; N, 6.04. Found: C, 66.41; H, 8.44; N, 5.98.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-*N*′-ϵ-2-chlorocarbobenzoxy-L-lysyl)amino]-5-(*N*′-octadecanoylamino)benzoic Acid (21). The compound **21** was synthesized from **18** (3.4 g) according to the similar procedures as described above: 2.2 g, 74% yield. ^{1}H NMR (DMSO-d_6) δ 10.10 (1H, s), 10.04 (1H, s), 8.20 (1H, s), 7.92 (2H, d), 7.47 (2H, d), 7.36 (2H, d), 6.99 (1H, d, J = 7.33 Hz, amide(Lys)), 5.07 (2H, s), 4.03 (1H, br), 3.00 (2H, br), 2.31 (2H, t), 1.58 (4H, br), 1.38 (11H, s), 1.22 (30H, br), 0.86 (3H, t). Anal. Calcd for $C_{44}H_{67}O_8N_4Cl$: C, 64.80; H, 8.28; N, 6.87. Found: C, 64.01; H, 8.98; N, 6.67.

3-[*N*-(*N*-*tert*-Butoxycarbonyl-*O*-benzyl-L-seryl)amino]-5-(*N*′-octadecanoylamino)benzoic Acid (22). The compound **22** was similarly synthesized from **19** (2.5 g): 1.7 g, 80% yield. ^{1}H NMR (DMSO-d_6) δ 10.23 (1H, s), 10.05 (1H, s), 8.21 (1H, s), 7.93 (1H, d), 7.90 (1H, d), 7.29 (5H, m), 7.04 (1H, br), 4.50 (2H, s), 4.38 (1H, br), 3.64 (2H, br), 2.30 (2H, t), 1.58 (2H, br), 1.39 (9H, s), 1.22 (28H, br), 0.84 (3H, t). Anal. Calcd for $C_{40}H_{61}O_7N_3$·2H$_2$O: C, 65.64; H, 8.95; N, 5.74. Found: C, 65.46; H, 8.63; N, 5.94.

Coupling and Cyclization of Peptides on Oxime Resin. The cyclic peptides (**1**−**4**, **23**, **24**, **25**) were synthesized by stepwise coupling of the corresponding dipeptides and further cyclization on oxime resin. The dry CH_2Cl_2 solution (18 cm^3) of the dipeptide (2 mmol) and DCC (2 mmol) was mixed with the oxime resin (2.0 g). The reaction mixture was allowed to shake at room temperature for 1 day. The solvent was filtered off, and the resin was washed with CH_2Cl_2 (× 2), CH_2Cl_2−C_2H_5OH (1:1 v/v) (× 2), and CH_2Cl_2 (× 2) and then dried in vacuo. The substitution level of the dipeptide to the oxime resin was estimated from the picrate assay; normally 0.23−0.28 mmol/g-resin.

Further coupling of dipeptides was performed as follows: the Boc-dipeptide-oxime resin, which was prepared as mentioned above, was washed with 25% TFA in CH_2Cl_2, and the resin was shaken in 25% TFA−CH_2Cl_2 at room temperature for 30 min. The solvent was filtered off, and the resin was washed with CH_2Cl_2 (× 2), 2-propanol (× 1), CH_2Cl_2 (× 2), and DMF (× 1). To the resin was added a DMF solution (18 mL) containing the dipeptide (3 equiv), [(benzotriazole-1-yl)oxy]tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) (3 equiv), HOBt (3 equiv), and triethylamine (5 equiv), and the resulted mixture was shaken at room temperature for 1 h. Then, the solvent was filtered off, and the resin was washed with DMF (× 2), and CH_2Cl_2 (× 2). The termination of the coupling reaction was checked by the Kaiser test. The same procedures were repeated to further couple the dipeptides.

To cyclize the peptides on the oxime resin, the Boc group was removed with 25% TFA−CH_2Cl_2. To the resulted peptide-bound resin, a DMF solution (18 mL) containing acetic acid (2 equiv of the peptides) and triethylamine (2 equiv) was added. The resin in the solution was shaken at room temperature for 1 day, the resultant mixture was filtered, and the resin was washed with DMF. The combined filtrate was evaporated in vacuo to give an oily residue, to which was added water to crystallize the peptides. Purification of the cyclic peptides was performed by chromatography over silica gel (Wakogel C-200) and Sephadex (LH-20), with $CHCl_3$:CH_3OH (30:1 v/v) and DMF as eluents, respectively. The yields and spectral data of the cyclic peptides are shown below.

Cyclo{-tri[3-(*N*-L-alanyl-amino)-5-(*N*′-decanoyl-amino)-phenylcarbonyl]-} (1): 17 mg, 2.6% yield, ^{1}H NMR (DMSO-d_6) δ 10.34 (3H, s), 10.05 (3H, s), 8.31 (3H, d, J = 7.32 Hz, amide(Ala)), 8.26 (3H, s), 7.98(3H, s), 7.61 (3H, s), 4.70 (3H, m), 2.33 (6H, t), 1.59 (6H, br), 1.44 (9H, d, J = 7.33 Hz, β-CH_3-(Ala)), 1.28 (36H, br), 0.87 (9H, t); ^{13}C NMR (DMSO-d_6) δ 171.6 (3C), 171.3 (3C), 166.1 (3C), 139.7 (3C), 139.2 (3C), 135.6 (3C), 113.5 (3C), 112.7 (3C), 112.1 (3C), 59.2 (3C), 36.4 (3C), 31.3 (3C), 28.6 (12C), 25.1 (3C), 22.1 (3C), 18.2 (3C), 14.0 (3C), MS m/z calcd for (M + Na) $C_{60}H_{87}O_9N_9$ 1101.6, found 1100.6. Anal.

Calcd for $C_{60}H_{87}O_9N_9 \cdot 2H_2O$: C, 64.66; H, 8.23; N, 11.31. Found: C, 64.62; H, 7.91; N, 11.08.

Cyclo{-tetra[3-(*N*-L-alanyl-amino)-5-(*N′*-decanoyl-amino)phenylcarbonyl]-} (2): 70 mg, 9% yield, ^{1}H NMR (DMSO-d_6) δ 10.10(4H, s), 10.01(4H, s), 8.47(4H, d, J = 6.83 Hz, amide(Ala)), 7.99(4H, s), 7.91(4H, s), 7.64(4H, s), 4.53(4H, q), 2.29(8H, t, J = 6.83 Hz, 2-alkanoyl), 1.59(8H, br), 1.40(12H, d, J = 7.33 Hz, β-CH_3), 1.25(48H, br), 0.83(12H, t, J = 6.35 Hz, 10-alkanoyl); ^{13}C NMR (DMSO-d_6) d 171.5 (8C), 166.6 (4C), 139.4 (4C), 139.1 (4C), 135.4 (4C), 113.6 (4C), 113.2 (4C), 112.8 (4C), 50.1 (4C), 37.7 (4C), 31.3 (4C), 28.9 (16C), 25.1 (4C), 22.1 (4C), 17.6 (4C), 13.9 (4C); MS m/z calcd for (M + Na) $C_{80}H_{116}O_{12}N_{12}$ 1461.1, found 1457.2. Anal. Calcd for $C_{80}H_{116}O_{12}N_{12} \cdot 4H_2O$: C, 63.63; H, 8.28; N, 11.13. Found: C, 63.95; H, 8.08; N, 11.22.

Cyclo{-tetra[3-(*N*-L-alanyl-amino)-5-(*N′*-palmitoyl-amino)phenylcarbonyl]-} (3): 177 mg, 17% yield, ^{1}H NMR (DMSO-d_6) δ 10.33−10.01(6H, m), 8.48−8.27(4H, m), 8.19−7.58(12H, m), 4.70−4.53(4H, m), 2.33−2.29(8H, m), 1.59(8H, br), 1.43(9H, br), 1.25(36H, br), 0.83(9H, t, J = 6.35 Hz, 10-alkanoyl); ^{13}C NMR (DMSO-d_6) δ 171.6 (8C), 166.6 (4C), 139.7 (4C), 139.2 (4C), 135.6 (4C), 113.6 (4C), 113.2 (4C), 112.7 (4C), 50.0 (4C), 38.9 (4C), 31.3 (4C), 29.0 (40C), 25.1 (4C), 22.1 (4C), 17.6 (4C), 13.9 (4C); MS m/z calcd for (M + Na) $C_{104}H_{164}O_{12}N_{12}$ 1797.5, found 1797.1. Anal. Calcd for $C_{104}H_{164}O_{12}N_{12} \cdot 5H_2O$: C, 66.99; H, 9.41; N, 9.02. Found: C, 66.97; H, 9.12; N, 9.34.

Cyclo{-penta[3-(*N*-L-alanyl-amino)-5-(*N′*-palmitoyl-amino)phenylcarbonyl]-} (4): 40 mg, 3.6% yield, ^{1}H NMR (DMSO-d_6) δ 10.16 (5H, s), 10.03 (5H, s), 8.53 (5H, br), 8.10−7.66(15H, br), 4.55 (5H, m), 2.29 (10H, br), 1.57 (10H, br), 1.40 (15H, d, J = 5.86 Hz, βCH_3), 1.22 (120H, br), 0.85 (15H, t). MS m/z calcd for (M + Na) $C_{130}H_{205}O_{15}N_{15}$ 2241.1, found 2240.7. Anal. Calcd for $C_{130}H_{205}O_{15}N_{15} \cdot 4H_2O$: C, 68.17; H, 9.38; N, 9.18. Found: C, 67.93; H, 9.30; N, 9.30.

Cyclo{-tetra[3-(*N*-(*O*-β-benzyl-L-aspartyl)amino)-5-(*N′*-palmitoyl-amino)phenyl carbonyl]-} (23): 35 mg, 3.2% yield, ^{1}H NMR (DMSO-d_6) δ 10.18 (4H, s), 10.01 (4H, s), 8.75 (4H, d), 8.09 (4H, s), 7.79 (4H, s), 7.70 (4H, s), 5.10 (8H, s), 4.99 (4H, m), 2.97 (8H, m), 2.29 (8H, br), 1.57 (8H, br), 1.22 (96H, br), 0.84 (12H, t) MS m/z calcd for (M + Na) $C_{136}H_{188}O_{20}N_{12}$ 2334.1, found 2333.1. Anal. Calcd for $C_{136}H_{188}O_{20}N_{12} \cdot 10H_2O$: C, 65.57; H, 8.42; N, 6.75. Found: C, 65.74; H, 8.03; N, 6.63.

Cyclo{-tetra[3-(*N*-(*N′*-ϵ-2-chlorocarbobenzoxy-l-lysyl)-amino)-5-(*N′*-stearoyl-amino)phenyl carbonyl]-} (24): 106 mg, 5.6% yield, ^{1}H NMR (DMSO-d_6) δ 10.38−10.00 (8H, br), 8.41−8.20 (4H, br), 8.20 (4H, s), 8.03 (4H, s), 7.96 (4H, s), 7.42 (8H, s), 7.32 (8H, d), 5.05 (8H, s), 4.50 (4H, br), 2.98 (8H, br), 2.29 (8H, br), 1.58 (16H, br), 1.44 (8H, br), 1.22 (120H, br), 0.85 (13H, t); MS m/z calcd for (M + Na) $C_{156}H_{228}O_{20}N_{16}Cl_4$ 2812.8, found 2808.5. Anal. Calcd for $C_{156}H_{228}O_{20}N_{16}Cl_4 \cdot 3H_2O$: C, 65.89; H, 8.30; N, 7.88. Found: C, 65.99; H, 8.26; N, 7.86.

Cyclo{-tetra[3-(*N*-(*O*-benzyl-l-seryl)amino)-5-(*N′*-stearoyl-amino)phenyl carbonyl]-} (25): 172 mg, 11% yield, ^{1}H NMR (DMSO-d_6) δ 10.52 (4H, s), 10.09 (4H, s), 8.40 (4H, s), 8.23 (4H, br), 8.00 (4H, s), 7.65 (4H, s), 7.29 (20H, m), 4.93 (4H, br), 4.55 (8H, s), 3.84 (8H, br), 2.32 (8H, t), 1.60 (8H, br), 1.22 (112H, br), 0.85 (12H, t); MS m/z calcd for (M + Na) $C_{140}H_{204}O_{16}N_{12}$ 2334.2, found 2333.5. Anal. Calcd for $C_{140}H_{204}O_{16}N_{12} \cdot 4H_2O$: C, 70.55; H, 8.97; N, 7.05. Found: C, 70.26; H, 8.96; N, 6.76.

Deprotection of the Cyclic Peptides 23−25. Removal of the protecting group, i.e., the benzyl group for aspartic acid and serine and the 2-chlorocarbobenzoxy group for lysine, in the cyclic peptides (**23**−**25**) was performed as follows. The protected cyclic peptides (30−60 mg) were dissolved in trifluoroacetic acid at −10 °C under N_2. To the solution, 3-cresol (0.06 cm^3), thioanisole (0.18 cm^3), and trimethylsilyl trifluoromethanesulfonate (TMSOTf; 0.28 cm^3) were added under N_2. The solution was sealed in a flask under N_2 and stirred at 0 °C for 1 h. The reaction solution was added to cold ether, and the mixture was allowed to stand in a refrigerator. The resultant powder was collected by centrifugation and was washed with ether. The solid was purified by chromatography on a Sephadex column (LH-20) with DMF.

Cyclo{-tetra[3-(*N*-l-aspartyl-amino)-5-(*N′*-hexadecanoyl-amino)phenylcarbonyl]-} (5). The compound was synthesized from **23** (30 mg) and was recrystallized from DMF−4% aqueous $NaHCO_3$ solution: 17 mg, 67% yield. ^{1}H NMR (DMSO-d_6) δ 10.25−10.01 (8H, m), 9.10−8.68 (4H, m), 8.07−7.35 (12H, m), 4.89 (4H, br), 2.95−2.67 (8H, br), 2.30 (8H, br), 1.58 (8H, br), 1.25 (96H, br), 0.85 (12H, t). MS m/z calcd for $C_{108}H_{164}O_{20}N_{12}$ 1950.6, found 1951.1. Anal. Calcd for $C_{108}H_{160}O_{20}N_{12}Na_4 \cdot H_2O$: C, 63.07; H, 7.94; N, 8.18. Found: C, 63.36; H, 8.11; N, 7.93.

Cyclo{-tetra[3-(*N*-l-lysyl-amino)-5-(*N′*-octadecanoyl-amino)phenylcarbonyl]-} (6). The compound was synthesized from **24** (50 mg). After purification by chromatography, trifluoroacetic acid and then ether was added to the eluted solution in order to obtain the solid as a TFA salt. The solid was recrystallized from DMF−water: 31 mg, 83% yield. ^{1}H NMR (DMSO-d_6) δ 10.38−10.03 (8H, br), 8.46−8.18 (4H, br), 8.04 (4H, s), 7.97 (4H, s), 7.84 (4H, s), 4.52 (4H, br), 2.89 (8H, br), 2.30 (8H, br), 1.58 (16H, br), 1.39 (8H, br), 1.23 (120H, br), 0.85 (13H, t); MS m/z calcd for $C_{124}H_{208}O_{12}N_{16}$ 2115.1, found 2114.3. Anal. Calcd for $C_{124}H_{208}O_{12}N_{16} \cdot 4(CF_3COOH) \cdot 10H_2O$: C, 57.62; H, 8.50; N, 8.15. Found: C, 57.30; H, 8.08; N, 8.17.

Cyclo{-tetra[3-(*N*-l-seryl-amino)-5-(*N′*-octadecanoylamino)phenylcarbonyl]-} (7). The compound was synthesized from **25** (60 mg) and was recrystallized from DMF-water: 44 mg, 87% yield. ^{1}H NMR (DMSO-d_6) δ 10.44−10.04 (8H, br), 8.89 (4H, br), 8.17−7.67 (12H, br), 4.66 (4H, br), 3.80 (8H, br), 2.30 (8H, br), 1.58 (8H, br), 1.23 (112H, br), 0.85 (12H, t); MS m/z calcd for (M + Na) $C_{112}H_{180}O_{16}N_{12}$ 1973.7, found 1973.2. Anal. Calcd for $C_{112}H_{180}O_{16}N_{12} \cdot 10H_2O$: C, 63.13; H, 9.46; N, 7.89. Found: C, 63.42; H, 8.78; N, 7.60.

Measurements of Single Channel Currents. Planar bilayers were formed by applying phosphatidylcholine (PC, asolectin type IV, Sigma Chemical Co., St. Louis, MO) solutions (40 mg/mL in n-decane) to a pore (300−500 μm in diameter) in a polypropylene partition separating two solution-filled chambers as described earlier.[78] A dimethyl sulfoxide (DMSO, Wako Co., Japan) solution of the peptide (1 mg/mL, ca. 0.5 mM) was premixed with the phospholipid solution (1:9, v/v) in a tube and was bath sonicated for 1 min to force the amount of peptide into the lipid solution as much as possible. Several minutes after sonication, the mixed solution automatically separated into two phases, where an n-decane solution formed an upper phase and a DMSO solution a lower phase. The bilayer membrane was formed from the n-decane solution, into which a certain amount of the peptide was expected to move. The same procedure for control experiment with DMSO only could not generate any channel activity in bilayers. To eliminate the possibility that the channel was formed from some contaminations in a high concentration of the peptide solution, we gradually lowered the concentration of the peptide in DMSO solution down to 1 μM, but no noticeable difference was observed for the single channel behavior. Curiously, the success rate of channel formation was only slightly improved by the high concentration of the peptide. Due to their poor solubility in lipid phase, it is suspected that most of the peptide molecules form an aggregation in the lipid solution such that they cannot participate in the formation of an ion channel. However, we used higher concentrations in most experiments to improve the success rate as much as possible.

Transmembrane currents across voltage-clamped bilayers were low-pass filtered at 10 kHz through a bilayer-clamp amplifier (model BC-525C, Warner Instrument Co. USA) and recorded on a magnetic tape by means of a PCM recorder (VR-10B, Instrutech Co.). The side to which lipid solution was smeared was defined as cis. The opposite side, which was connected to the signal ground of the amplifier, was defined as trans. The voltage was referred to the cis side with respect to the trans side. All the solutions used were buffered by 5 mM HEPES and adjusted with Tris base to pH 7.2. All the experiments were carried out at room temperature (20−25 °C).

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Data Analyses. Single channel currents recorded on the tape were replayed, postfiltered at 0.10 kHz, and digitized at 0.50 ms/point by means of an analogue to digital converter (model 2801A, Data Translation Co.). Amplitude of the single channel currents was measured as a peak-to-peak distance (each peak represents open and closed levels) on the amplitude histogram constructed by a single channel analysis program, PAT (ver. 6.2).[79] Single channel conductance values at a symmetrical salt solution were determined by the slope of the current−voltage ($I-V$) curve, where the $I-V$ data could be well fitted to a straight line. Single-channel conductance values under asymmetrical ionic conditions were obtained by the slope of $I-V$ curves at higher voltages (≥60 mV), where nonlinear $I-V$ curves became asymptotic to straight lines. The permeability ratios of the channel (P_{Na^+}/P_{K^+}) were defiened as

$$P_{Na^+}/P_{K^+} = (a_{K^+}/a_{Na^+})\exp(V_r F/RT) \quad (3)$$

under biionic conditions,

$$\frac{P_{Cl^-}}{P_{K^+}} = \frac{[a_{K^+}]_c - [a_{K^+}]_t \exp(-V_r F/RT)}{[a_{Cl^-}]_c \exp(-V_r F/RT) - [a_{Cl^-}]_t} \quad (4)$$

under asymmetrical conditions,

where the reversal potential V_r was obtained by fitting the $I-V$ curve to the Goldman−Hodgkin−Katz (GHK) voltage equation,[1] a_{K^+} and a_{Na^+} are the activity of K^+ and Na^+, subscripts c and t represent cis and trans sides of the chamber, F is the Faraday constant, R is the gas constant, and T means absolute temperature.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for $(AC_{10})_3$ and $(AC_{10})_4$; temperature dependent ^{1}H NMR for $(AC_{10})_3$, $(AC_{10})_4$, and $(AC_{16})_5$; NMR peak assignments for all compounds except for **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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