JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
pubs.acs.org/JACS

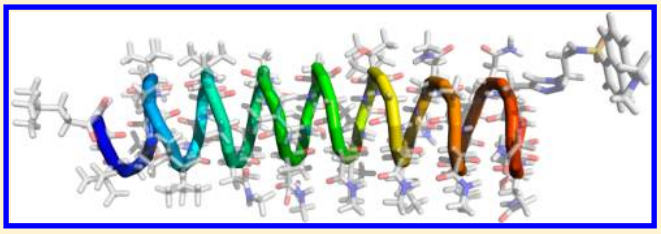
Design, Synthesis and Functional Analysis of Dansylated Polytheonamide Mimic: An Artificial Peptide Ion Channel

Hiroaki Itoh,[†] Shigeru Matsuoka,[†] Mohamed Kreir,[‡] and Masayuki Inoue*[,†]

[†]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[‡]Nanion Technologies GmbH, Gabrielenstrasse 9, D-80636 Munich, Germany

Supporting Information

ABSTRACT: We report herein the design, total synthesis, and functional analysis of a novel artificial ion channel molecule, designated as dansylated polytheonamide mimic (**3**). The channel **3** was designed based on an exceptionally potent cytotoxin, polytheonamide B (**1**). Our strategy for the development of synthetic ion channels, which could be easily derivatized for various functions, involved two key features. First, the structure of **1** was simplified by replacing many of nonproteinogenic amino acid residues which required multistep synthesis by commercially available amino acids while retaining those residues necessary for folding. It significantly reduced the number of synthetic steps and facilitated a practical chemical construction of **3**. Second, the introduction of propargyl glycine at residue 44 enabled facile installation of dansyl group as a reporter of the membrane localization of **3**. Application of a newly designed protective group strategy provided efficient construction of the 37 amino acid sequence of residues 12−48 through one automatic solid-phase peptide synthesis. After peptide cleavage from the resin, **3** was synthesized via dansyl group introduction and one fragment-coupling reaction with residues 1−11, followed by the global deprotection. The simplified mimic **3** exhibited potent cytotoxicity toward p388 mouse leukemia cells (IC_{50} = 12 nM), effectively induced ion transport across the lipid bilayers of liposomes, and displayed H^+ and Na^+ ion channel activities. Because of its simplified yet functional scaffold structure with a potential for diversification, our rationally designed ion channel molecule should be useful as a novel platform for developing various cytotoxic channel molecules with additional desired functions.



INTRODUCTION

Peptides biosynthesized by nonribosomal peptide synthetases are one of the most important classes of natural products.[1] As broad as the spectrum of biological activities, so is the structural diversity of these peptides, which are mostly cyclic or branched cyclic compounds containing nonproteinogenic amino acids, small heterocyclic rings, and other unusual modifications in the peptide backbone. Several nonribosomal peptides such as gramicidin A[2] and polytheonamide B (**1**, Figure 1)[3] have linear sequences of alternating D- and L-chirality. These peptides fold into three-dimensional structures distinct from ribosomal proteins, and these specific shapes relate to their biological activities.[1]

Polytheonamide B (**1**), which possesses 48 amino acid residues, is known to be the largest nonribosomal peptide, and its component monomers are 6 proteinogenic and 13 nonproteinogenic amino acids. Extensive NMR studies in $CHCl_3$/MeOH solution revealed that **1** adopts a $\beta^{6.3}$-helix structure with a length of approximately 45 Å and a hydrophilic pore with a diameter of 4 Å.[4] Since ion channel formation through a $\beta^{6.3}$-helical conformation is known for a head-to-head dimer of gramicidin A of 15 residues,[5] the 3-fold larger helix of monomeric **1** is believed to function as a transmembrane channel by itself. In fact, single channel recordings demonstrated that **1** formed a monovalent cation channel for H^+, Na^+, K^+, Rb^+ and Cs^+.[6] Compound **1** was also shown to be approximately 50 times more cytotoxic against P388 mouse leukemia cells (IC_{50} = 0.098 nM) than gramicidin (IC_{50} = 4.3 nM, see Supporting Information), and this exceedingly high toxicity can be attributed to the ability of **1** to efficiently form a highly stable ion channel. The unique three-dimensional shape, ion channel functions and cytotoxicity of **1** are attributable to the unusual constituent amino acids and their specific sequential arrangements.

Motivated by its unique functions and synthetically challenging structure, we launched a program to construct **1**, and reported its first total synthesis in 2010.[7,8] Our synthesis of 161 steps involved four independent stages: synthesis of the nonproteinogenic amino acids (58 steps), automatic solid-phase assembly of four fragments of 7 to 16 residues (residues 1−11, 12−25, 26−32, 33−48, 94 steps), solution-phase functionalization and condensation of the fragments (8 steps), and global deprotection (1 step). Subsequent structure−activity relationship studies demonstrated the importance of the overall length and the hydrophobic N-terminal region of **1** for its cytotoxicity and ion channel

Received: April 21, 2012
Published: August 4, 2012

dx.doi.org/10.1021/ja303831a | J. Am. Chem. Soc. 2012, 134, 14011−14018

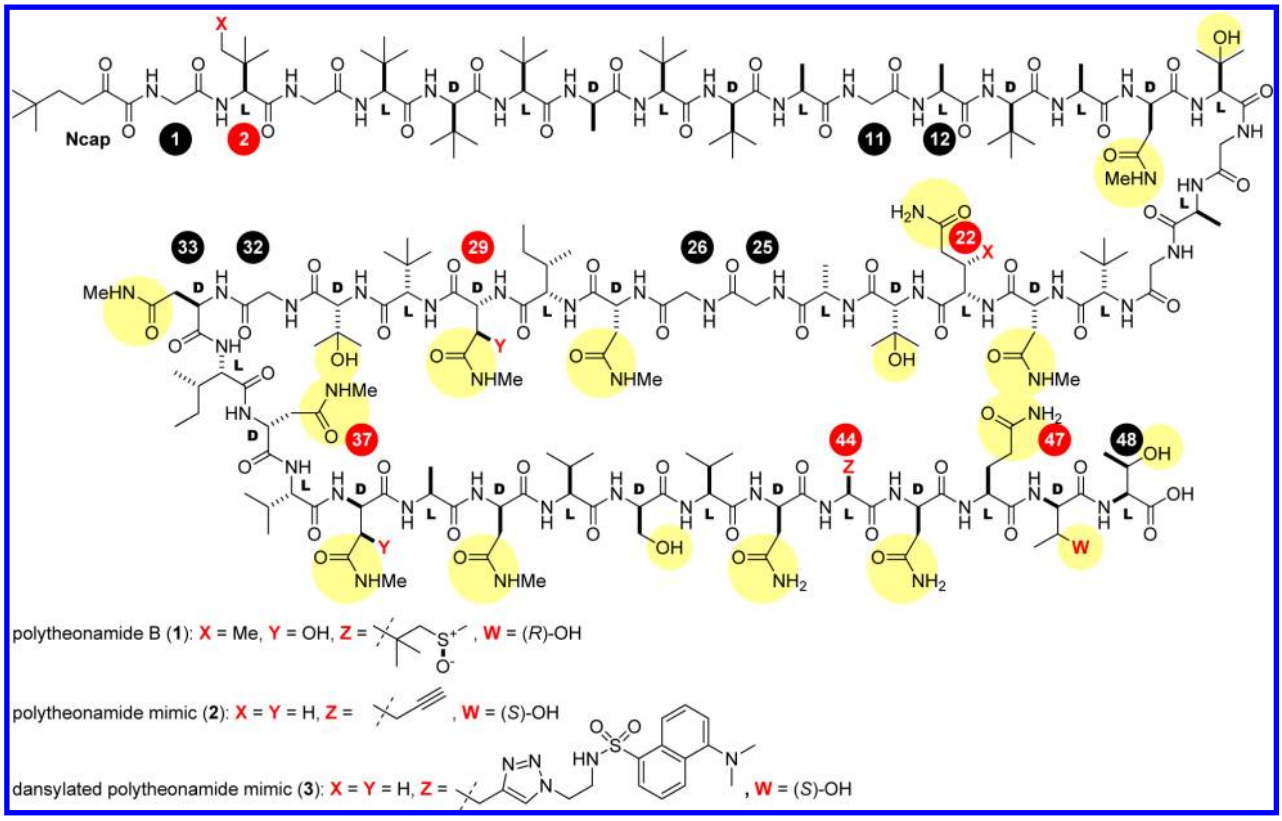


Figure 1. Structures of polytheonamide B and dansylated polytheonamide mimic. Residue numbers indicated in red are the amino acid residues artificially modified from natural polytheonamide B. The potential functional groups involved in the hydrogen bonding network between the side chains are highlighted in yellow.

activity.[9] These accomplishments provided valuable information on the structural elements relating to the functions of **1**.

For performing further extensive and systematic biological studies, however, it is particularly important to supply polytheonamide B structures in a more practical fashion. Accordingly, we decided to design new and simplified polytheonamide B analogues that would retain the functions of the original natural product and would be prepared by shorter and more automated routes. Here, we report the synthesis of dansylated polytheonamide mimic **3** (Figure 1), which requires significantly less synthetic steps (127 steps for **3** vs 161 steps for **1**), consisting of minimized solution-phase reactions (31 steps) and maximized automated solid-phase condensations (96 steps). The simplified mimic **3** exhibited potent cytotoxicity, effectively induced ion transportation across lipid bilayers and displayed ion channel activity, suggesting that our rationally designed peptide structure will serve as a novel platform for creating tailor-made cytotoxic molecules and artificial transmembrane ion channels.[10]

RESULTS AND DISCUSSION

Multiple chemical modulations of the residues in natural peptide sequences often lead to loss of their original biological activity, because the structures and order of their constituent amino acids encode their functions. Depending on peptide length and residue composition, linear D,L-peptides can in fact adopt diverse single-stranded or double-stranded β-helices of various pore sizes.[10k,11−13] Therefore, judicious incorporation of the function-determining features of **1** was crucial for simplifying the entire structure of **1**.

The $\beta^{6.3}$-helix formation of polytheonamide B (**1**) is considered to be stabilized by hydrogen bonding interactions not only of the main chain, but also of the side chains of the residues. The hydroxy and amide groups of the side chains highlighted in Figure 1 are proposed to be involved in the hydrogen bond network that reinforces the $\beta^{6.3}$-helix of **1**.[4,14] Therefore, we hypothesized that retaining the interresidue hydrogen bonding of both the main and side chains would be essential for mimicking the structure and function of **1**.

These considerations guided us in the design of the synthetically more accessible polytheonamide mimic (**2**, Figure 1). While maintaining alternating D- and L-α-chiral centers of the main chain of **1** and the hydroxy and amide groups of the side chains, the β-substituents of residues 2, 22, 29, and 37 were removed and residue 47 was changed from D-*allo*-threonine to D-threonine. These minimum modifications significantly reduced the number of steps for preparation of the amino acid monomers. Additionally, the 44th amino acid, β,β-dimethyl L-methionine oxide, was replaced by L-propargyl glycine for installing a variety of azide molecules via 1,3-dipolar coupling reactions. This particular alteration would enable the incorporation of various probes and controlling elements at the last stage of the synthesis through click chemistry.[15] In this work, the dansylated polytheonamide mimic (**3**) was selected as the target molecule, because the fluorescent dansyl group could be utilized as a probe for investigation of the physicochemical behavior of **3** under biological settings. Prior to the synthesis, a three-dimensional model of **3** was generated based on the NMR structure of **1** (Figure 2), indicating that the main and side chain hydrogen bond networks of **1** were preserved.

dx.doi.org/10.1021/ja303831a | J. Am. Chem. Soc. 2012, 134, 14011−14018

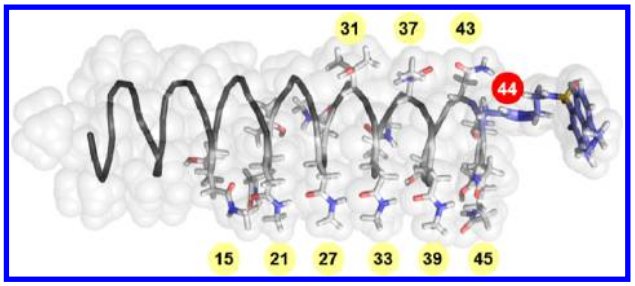


Figure 2. Computer-generated $\beta^{6.3}$-helical structure model of dansylated polytheonamide mimic (**3**). The three-dimensional structure was generated based on the NMR structure of polytheonamide B (PDB 1D code: 2RQO). The side chains that potentially participate in a hydrogen bonding network along the helix axis are shown in the standard atom colors. The side chain of residue 44 is also shown.

Intriguingly, the dansyl group of residue 44 was positioned close to the pore entrance of the helical structure in this model.

Component amino acids and structural units used for the total synthesis of **3** are illustrated in Figure 3. Importantly, all the units except **4** (Ncap−OH), **L-10**, **D-10**, **D-15**, and **L-16** were commercially available, and thus, the number of steps for their preparations were significantly reduced in comparison to

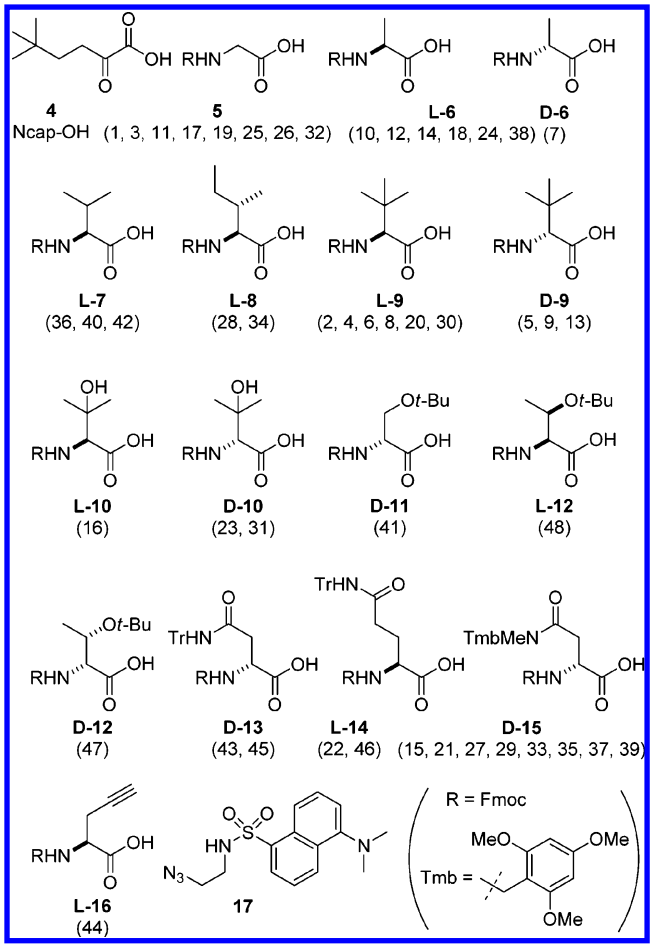
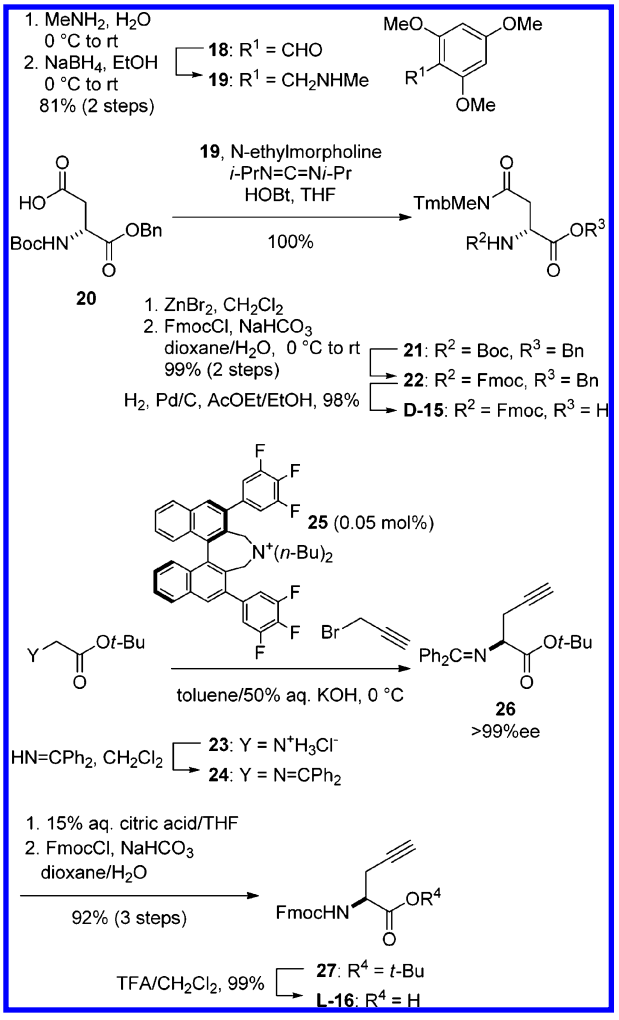


Figure 3. Component amino acids and structural units used for total synthesis of dansylated polytheonamide mimic (**3**). The residue numbers are indicated in parentheses. *t*-Bu = *t*-butyl; Fmoc = 9-fluorenylmethoxycarbonyl; Tmb = 2,4,6-trimethoxybenzyl; Tr = triphenylmethyl.

those for **1** (27 steps for **3** vs 58 steps for **1**). The Fmoc (9-fluorenylmethoxycarbonyl) group was used for protection of the α-nitrogens of the amino acids, and Tr (triphenylmethyl), Tmb (2,4,6-trimethoxybenzyl group),[16] and *t*-Bu groups were applied for capping the primary and secondary amides, and the hydroxy groups, respectively, of the side chains. Although Tmb was not used in the total synthesis of **1**,[7] it was envisaged that this group would be simultaneously removed with the two other protective groups (Tr and *t*-Bu) upon acid activation at the last step of the total synthesis of **3**.

Syntheses of compounds **4** (4 steps), **L-10** (6 steps), and **D-10** (6 steps) were previously reported,[7] and preparations of **D-15** and **L-16** were realized from the commercially available compounds in 6 and 5 steps, respectively, in high overall yields (Scheme 1). Reductive methylamination of 2,4,6-trimethoxybenzaldehyde **18** led to **19**, which was condensed with the D-aspartic acid derivative **20** to produce **21**. Chemoselective removal of the Boc group in the presence of the acid-labile Tmb group was realized using $ZnBr_2$,[17] and the liberated amine was protected to generate the Nα-Fmoc **22**. The last hydrogenolysis of the Bn ester transformed **22** into **D-15**. Synthesis of **L-16** started from Schiff base formation from glycine *tert*-butyl ester **23**. The generated **24** was subjected to

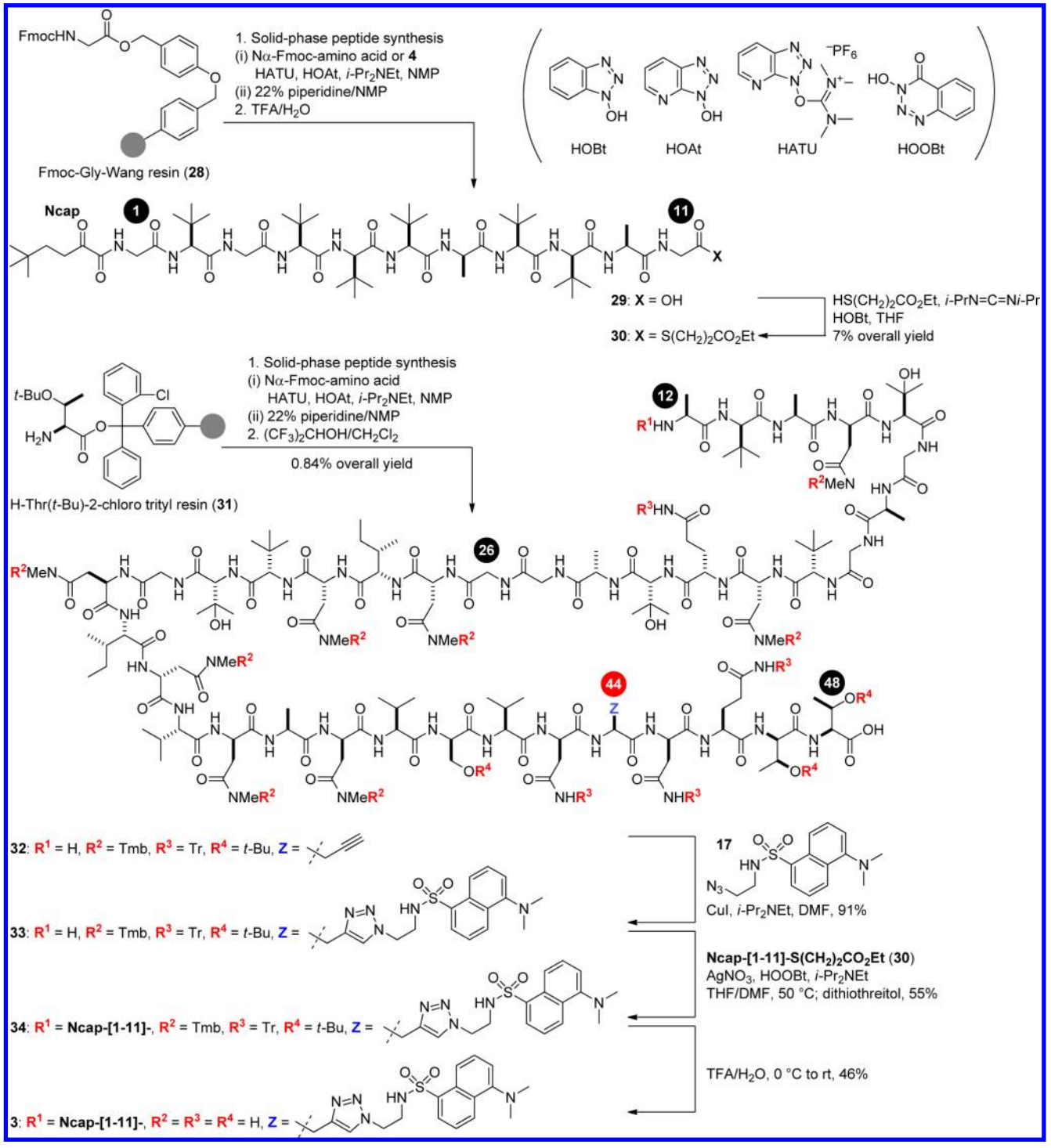
Scheme 1. Syntheses of Two Fmoc-Protected Amino Acids D-15 and L-16[a]



[a]HOBt = 1-hydroxybenzotriazole; TFA = trifluoroacetic acid.

dx.doi.org/10.1021/ja303831a | J. Am. Chem. Soc. 2012, 134, 14011−14018

Scheme 2. Total Synthesis of Dansylated Polytheonamide Mimic (3)[a]



[a]HATU = *O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt = 1-hydroxy-7-azabenzotriazole; HOOBt = 3,4-dihydro-3-hydro-4-oxo-1,2,3-benzotriazine.

phase transfer catalytic alkylation with propargyl bromide in the presence of Maruoka catalyst **25**,[18] leading to enantiomerically pure **26**. Finally, hydrolysis of the imine of **26**, followed by Fmoc protection, afforded **27**, which was converted to carboxylic acid **L-16** using TFA.

Having synthesized the requisite units, the component amino acids were condensed using a solid-phase peptide synthesizer (Scheme 2). To maximize the automated elongation, the sequence of dansylated polytheonamide mimic (**3**) was divided into two segments: residues 1−11 (**30**) and residues 12−48 (**33**). We planned to synthesize the N-terminal residues 1−11 (**30**) separately and then couple **30** with residues 12−48 (**33**), because a segment coupling between **30** and **33** at the reactive 11th glycine would give the full sequence more efficiently than stepwise attachments of six sterically demanding *β*-methyl valines to **33** through solid-phase synthesis. Accordingly, the N-

dx.doi.org/10.1021/ja303831a | J. Am. Chem. Soc. 2012, 134, 14011−14018

terminal segment was synthesized from Fmoc-Gly-Wang resin[19] using Fmoc-based chemistry[20] with HATU/HOAt[21] activation. After cleavage from the Wang resin by treatment with 95% aqueous TFA, the obtained **29** was converted to the corresponding thioester **30** using the reagent combination of $HS(CH_2)_2CO_2Et$, HOBt, and *N*,*N*′-diisopropylcarbodiimide (7% overall yield).[22]

Condensation of 37 amino acids of residues 12−48 of **3** turned out to be no easy task. To achieve this, a protective group strategy was redesigned from the total synthesis of polytheonamide B (**1**),[7] in which only 16 amino acids (residues 33−48) were automatically elongated in a reasonable yield. After many experiments, it became clear that protections of the CONHMe groups in addition to the $CONH_2$ and OH moieties were crucial for the high-yielding synthesis. As suggested from the modeling studies (see Figure 2),[4,14] the CONHMe groups act as hydrogen bond donor/acceptor, thus could facilitate the formation of nonreactive aggregates in the peptide-resin matrix. Specifically, absence of the Tmb group on the methyl amide groups of the corresponding residues decreased the synthetic efficiency even when the Tr and *t*-Bu groups were present: elongation of the peptide was impeded beyond residue 26 when the de-Tmb version of **D-15** was used.[23] In contrast, the Tmb-protected peptide of 37 residues was successfully synthesized by the action of the HATU/HOAt reagent system from H-Thr(*t*-Bu)-2-chloro trityl resin[24] including the eight condensations of **D-15**. Next, cleavage from the 2-chloro trityl resin was achieved under mild acidic conditions [$(CF_3)_2CHOH$ and CH_2Cl_2] without deprotecting the side chains, giving rise to **32** in 0.84% overall yield. As far as we know, **32** is the longest D,L-alternating sequence prepared to date through the stepwise solid-phase synthesis. Upon treatment with azide **17**[25] in the presence CuI and *i*-Pr_2NEt,[26] thus obtained segment **32** underwent smooth 1,3-dipolar addition to produce the dansylated compound **33**.

After syntheses of the two segments **30** and **33**, only two reactions, coupling and global deprotection, remained to be established for the total synthesis of **3** (Scheme 2). Careful optimization of the Ag^+-mediated conditions[27] was required for the coupling, even though the reaction had been successfully applied to the total synthesis of **1**. When thioester **30** and amine **33** were treated with $AgNO_3$ and HOOBt in DMF at 50 °C, the full-length polyamide **34** was obtained in 55% yield after addition of dithiothreitol and subsequent HPLC purification. It is noteworthy that use of dithiothreitol[28] as a scavenger of the residual silver salt before purification was critical for reproducible yields, suggesting that the dansyl-connected triazole group captured Ag^+ to form a stable chelate.[29]

The last reaction of the synthesis was simultaneous removal of the eight Tmb, four Tr and three *t*-Bu groups from **34** (Scheme 2). This challenging reaction was realized by carefully tuning acidic conditions, temperature and time. Exposure of **34** to a TFA/H_2O (95:5) mixture at 0 °C to room temperature for 2 h and subsequent HPLC purification delivered dansylated polytheonamide mimic **3** in 46% yield. The primary structure of **3** was determined by DQF-COSY, TOCSY, NOESY and $^1H-^{13}C$ HSQC experiments (see Supporting Information for details). Hence, the simplified mimic **3** was synthesized via a considerably more practical and efficient route in comparison to the 161-step synthesis of original **1**. The total 127 steps comprised 27 steps of monomer syntheses, 96 steps of automated solid-phase syntheses and only 4 steps of technically demanding reactions of the large peptides, demonstrating the high operational superiority of construction of **3**.

Completion of the synthesis of **3** enabled biological and biophysical studies to be performed. A cytotoxicity assay against p388 mouse leukemia cells was performed using the XTT method.[30] Compound **3** exhibited strong cytotoxicity with an IC_{50} value of 12 nM. Although the toxicity of **3** was approximately 100-times weaker than that of polytheonamide B (**1**) (IC_{50} = 0.098 nM), it was remarkable that multiple structural modifications at six residues of **1** did not result in complete loss of toxicity, indicating that our designed molecule maintained the activity-determining structural features of **1**.

To induce ion current across the membrane, it should be a prerequisite that the ion entrances of the N- and C-terminal regions of **3** are situated in an aqueous environment. The absorption spectra of the solvatochromic dansyl group[31] of **3** provided important information on the position of residue 44 within a phospholipid bilayer of liposomes. Namely, the microenvironment of the fluorophore in **3** was analyzed by measuring the Stokes shift (*S*) (Table 1), which was calculated

Table 1. Excitation ($\lambda_{max,ex}$) and Emission ($\lambda_{max,em}$) Maximum Wavelengths of 3, and Its Stokes Shift Values (*S*)[a]

environment	$\lambda_{max,ex}$ (nm)	$\lambda_{max,em}$ (nm)	*S* (cm^{-1})
n-BuOH	343	511	9585
EtOH	342	514	9785
MeOH	344	528	10130
buffer	330	540	11785
EYPC LUVs	341	533	10564

[a] *S* is defined by $S = 1/\lambda_{max,ex} - 1/\lambda_{max,em}$, where $\lambda_{max,ex}$ and $\lambda_{max,em}$ are the wavelengths (in cm) of the maximum of the excitation and the emission spectra, respectively. Buffer = pH 7.2, 10 mM sodium phosphate, 100 mM NaCl buffer; EYPC = egg yolk phosphatidyl choline; LUV = large unilamellar vesicle.

from the wavelengths of the maximum of the excitation and emission spectra. The *S* values were determined for **3** in a range of solvents (*n*-butanol, ethanol, methanol, and sodium phosphate buffer), showing their strong dependence on the polarity of the medium. Peptide **3** was then introduced into liposomes consisting of EYPC (egg yolk phosphatidylcholine), and its *S* value (10 564 cm^{-1}) was determined to be close to that in methanol (10 130 cm^{-1}). Since the polarity in the near-surface region of the membrane is known to be comparable to that in methanol,[32] the dansyl group of the C-terminal moiety was found to be exposed to the headgroup domain of the phosphatidylcholine bilayer as expected from its requisite proximity to the channel entrance.[33]

The next set of liposome experiments proved that mimic **3** indeed exhibited efficient ion transport activity without disruption of the membrane.[34] First, lack of the membrane-disruption activity of **3** was confirmed using the assay of liposomes (EYPC/cholesterol = 2:1) encapsulating carboxy-fluorescein (CF, 20 mM).[35] The fluorescence of highly concentrated CF molecules was suppressed by self-quenching before detergent-like molecules released CFs to increase the fluorescence. As shown in Figure 4a, mimic **3** had no effect on the CF leakages, similar to the parent compound **1**. Second, the ion transport activity was evaluated by applying a pH gradient across the membrane. The liposomes were prepared in HEPES buffer containing 200 mM NaCl with a pH gradient, a pH of 6.5 inside the liposome and a pH of 7.5 outside. Pyranine

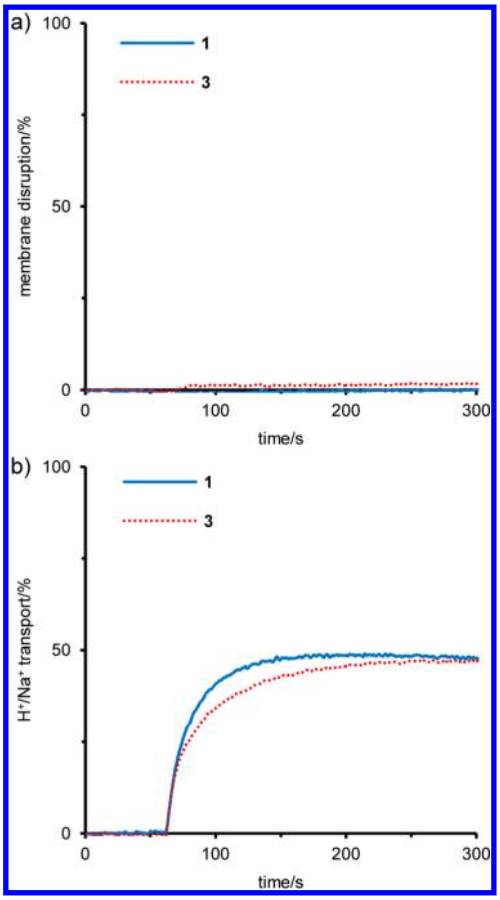


Figure 4. Evaluation of membrane-disrupting activity and ion transport activity of polytheonamide B (**1**) and dansylated polytheonamide mimic (**3**) using liposomes. (a) Time-course of CF leakage from LUVs (EYPC/cholesterol = 2:1) caused by **1** and **3**. CF leakage by each peptide was determined by fluorescence intensity of leaked CF standardized against the maximum leakage by Triton X-100. (b) Time-course of H^+/Na^+ exchange across lipid bilayers of pH-gradient LUVs (EYPC/cholesterol = 2:1) caused by **1** and **3**. The ion transport was evaluated as the pH-dependent fluorescence from pyranine standardized against the maximum exchange by Triton X-100. In all the experiments, **1** or **3** was added at 60 s. CF = carboxyfluorescein; pyranine = trisodium 8-hydroxypyrene-1,3,6-trisulfonate.

(trisodium 8-hydroxypyrene-1,3,6-trisulfonate, 1 mM) was introduced into the liposomes as a pH indicator.[36] After addition of the peptide (125 nM), the transport activity of alkali ions was characterized over time from the fluorescence increase by deprotonation of the phenolic proton of pyranine. In these experiments, the peptide channels allowed exchange between protons and Na^+ ions across the lipid bilayer of the vesicles to preserve the ionic milieu. As shown in Figure 4b, polytheonamide B (**1**) and **3** both induced a rapid increase of the fluorescence until it reached a plateau, although the ion transport by **3** was slightly less efficient than that by **1**.[37] Nevertheless, mimic **3** successfully emulated the facile ion transportation of original **1**.

To further demonstrate that **3** forms ion channels for Na^+ ions and protons, and to compare its channel behavior to that of **1**, single channel recording experiments were performed in NaCl and HCl solutions.[38] A planar lipid bilayer consisting of DPhPC (diphytanoyl phosphatidylcholine) was formed either in neutral solution (1 M NaCl, pH 7.4) or in acidic aqueous solution (0.1 M HCl, pH 1.0), and then Na^+ or H^+ current across the membrane was measured.[39] Similar to **1**,[40] the apparent single-channel behavior with open and closed states appeared immediately after addition of **3** under both the neutral (Figure 5a) and acidic conditions (Figure 5b). Most interestingly, whereas the shapes of the Na^+ current–voltage curves of **1** and **3** were comparable (Figure 5c), their H^+ current–voltage relations were found to be significantly different (Figure 5d). Considering the similar activities of **1** and **3** as the Na^+ channels as well as their same H^+/Na^+ ion exchange activities in the liposome experiments (see Figure 4b), much smaller H^+ conductance associated with **3** (30–40 pS) than that of **1** (120–150 pS) can be attributed to their distinct experimental conditions. Namely, the lower pH necessary for the H^+ channel experiments (pH 1) in comparison to the Na^+ channel (pH 7.4) and liposome experiments (pH 6.5–7.5) would induce the functional change of **3**. Since the dansyl group would be located in the water/lipid interface according to the solvatochromism study, it is likely that the basic amine of the dansyl group[41] undergoes protonation from the aqueous phase at pH 1. The protonated cationic amine could in turn lower the channel activities through binding to the ion entrance or by inducing a repulsive electrostatic effect toward incoming protons.[42,43] This suggests an exciting possibility of pH-driven control of the channel functions of **3**, though future detailed studies would be necessary to further prove the hypothesis.

■ CONCLUSIONS

In conclusion, we have accomplished the design, total synthesis and functional analysis of dansylated polytheonamide mimic (**3**), an artificial ion channel. In designing **3**, the sequence of the original natural product polytheonamide B (**1**) was simplified by changing the β-substituents of residues 2, 22, 29, 37, and 47 and replacing the β,β-dimethyl L-methionine oxide of residue 44 with L-propargyl glycine. After preparation of the monomer units for **3**, application of the new protective group strategy enabled construction of the 37 amino acid sequence through one automatic solid-phase synthesis and peptide cleavage from the resin. Then, the 48-residue sequence **3** was synthesized in three steps: the dansyl group introduction, the one fragment-coupling reaction and the global deprotection. The highly automated synthetic route to **3** required significantly less synthetic steps in comparison to original **1** (127 steps vs 161 steps). Despite the multiple substitutions, **3** successfully emulated the cytotoxic and ion channel activities of **1**. Peptide **3** exhibited potent cytotoxicity toward P388 cells, effectively induced H^+/Na^+ exchange across the lipid bilayers of liposomes, and displayed the Na^+ and H^+ ion channel activities. Furthermore, the fluorescent dansyl group permitted us to reveal its microenvironment within the membrane, demonstrating an advantage of mimic **3** over **1** for studying physicochemical behaviors of the peptide channel. Because of practicality in total synthesis and efficiency in introduction of diverse functional structures via click chemistry, our polytheonamide mimic will serve as a novel platform for optimizing and controlling cytotoxic and channel activities and for bestowing artificial functions that the original natural product does not possess.

dx.doi.org/10.1021/ja303831a | J. Am. Chem. Soc. 2012, 134, 14011–14018

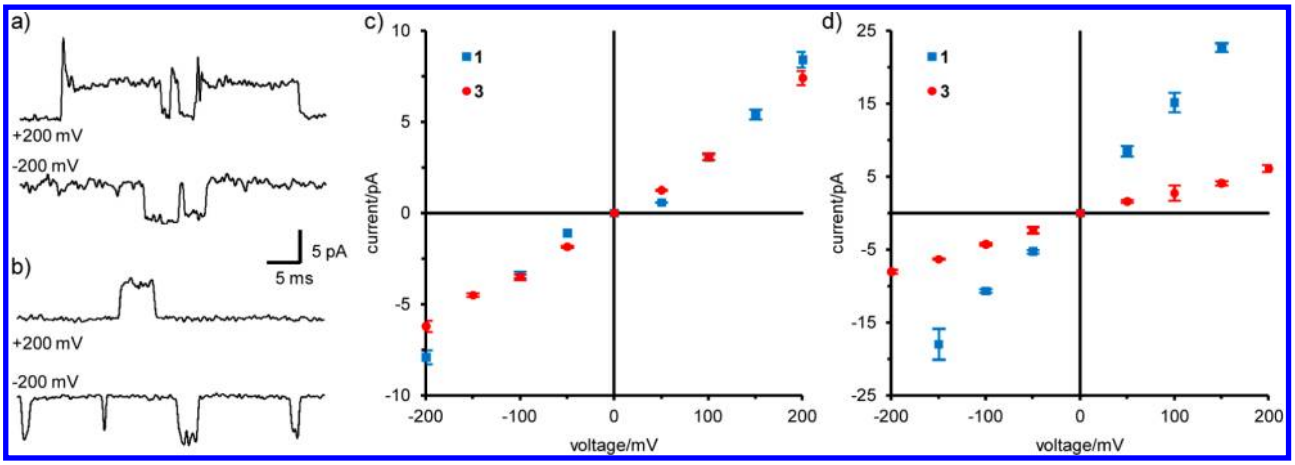


Figure 5. Single-channel current recording on the Port-a-Patch patch clamp system for **1** and **3**. (a) Traces showing Na^+ current flow through a planar DPhPC bilayer in 1 M NaCl (pH 7.4) containing a channel formed by **3**. (b) Traces showing H^+ current flow through a planar DPhPC bilayer in 0.1 M HCl (pH 1.0) containing a channel formed by **3**. (c) Current−voltage (I−V) curves were obtained for **1** and **3** from Na^+ channels observed in the current traces. The Na^+ conductance values were 52 pS (**1**, 50−200 mV), 45 pS (**1**, −50 ∼ −200 mV), 41 pS (**3**, 50−200 mV), and 28 pS (**3**, −50 ∼ −200 mV). (d) Current−voltage (I−V) curves were obtained for **1** and **3** from H^+ channels observed in the current traces. The H^+ conductance values were 153 pS (**1**, 0−150 mV), 115 pS (**1**, 0 ∼ −150 mV), 29 pS (**3**, 0−200 mV), and 40 pS (**3**, 0 ∼ −200 mV). DPhPC = diphytanoyl phosphatidylcholine.

■ ASSOCIATED CONTENT

Supporting Information

Characterization data for all new compounds, experimental procedures, and assay data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

inoue@mol.f.u-tokyo.ac.jp

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported financially by Funding Program for Next Generation World-Leading Researchers (JSPS) to M.I. Fellowship from JSPS to H.I. is gratefully acknowledged. We thank Dr. Fumiaki Hayashi (RIKEN SSBC, Yokohama, Japan) for measurement of 800 MHz ^{1}H NMR spectra.

■ REFERENCES

(1) For reviews of nonribosomal peptides, see: (a) Caboche, S.; Pupin, M.; Leclère, V.; Fontaine, A.; Jacques, P.; Kucherov, G. *Nucleic Acids Res.* **2008**, *36*, D326. (b) Felnagle, E. A.; Jackson, E. E.; Chan, Y. A.; Podevels, A. M.; Berti, A. D.; McMahon, M. D.; Thomas, M. G. *Mol. Pharmaceutics* **2008**, *5*, 191. (c) Sieber, S. A.; Marahiel, M. A. *Chem. Rev.* **2005**, *105*, 715. (d) Finking, R.; Marahiel, M. A. *Annu. Rev. Microbiol.* **2004**, *58*, 453. (e) Schwarzer, D.; Finking, R.; Marahiel, M. A. *Nat. Prod. Rep.* **2003**, *20*, 275.

(2) Hotchkiss, R. D.; Dubos, R. J. *J. Biol. Chem.* **1940**, *132*, 791.

(3) (a) Hamada, T.; Matsunaga, S.; Yano, G.; Fusetani, N. *J. Am. Chem. Soc.* **2005**, *127*, 110. (b) Hamada, T.; Sugawara, T.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1994**, *35*, 719.

(4) Hamada, T.; Matsunaga, S.; Fujiwara, M.; Fujita, K.; Hirota, H.; Schmucki, R.; Güntert, P.; Fusetani, N. *J. Am. Chem. Soc.* **2010**, *132*, 12941.

(5) (a) Wallace, B. A. *J. Struct. Biol.* **1998**, *121*, 123. (b) Ketchem, R. R.; Hu, W.; Cross, T. A. *Science* **1993**, *261*, 1457. (c) Killian, J. A. *Biochim. Biophys. Acta* **1992**, *1113*, 391. (d) Stankovic, C. J.; Heinemann, S. H.; Delfino, J. M.; Sigworth, F. J.; Schreiber, S. L. *Science* **1989**, *244*, 813. (e) Wallace, B. A.; Ravikumar, K. *Science* **1988**, *241*, 182. (f) Urry, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 672. For a review, see: (g) *Gramicidin and Related Ion Channel-Forming Peptides*; Wiley & Sons: Chichester, 1999. For recent applications of gramicidin channel, see: (h) Lundbæk, J. A.; Collingwood, S. A.; Ingólfsson, H. I.; Kapoor, R.; Andersen, O. S. *J. R. Soc., Interface* **2010**, *7*, 373. (i) Majd, S.; Yusko, E. C.; Billeh, Y. N.; Macrae, M. X.; Yang, J.; Mayer, M. *Curr. Opin. Biotechnol.* **2010**, *21*, 439. (j) Majd, S.; Yusko, E. C.; MacBriar, A. D.; Yang, J.; Mayer, M. *J. Am. Chem. Soc.* **2009**, *131*, 16119. (k) Macrae, M. X.; Blake, S.; Jiang, X.; Capone, R.; Estes, D. J.; Mayer, M.; Yang, J. *ACS Nano* **2009**, *3*, 3567. (l) Wesolowski, R.; Sommer, A.; Arndt, H.-D.; Koert, U.; Reiß, P.; Wimmers, S.; Strauss, O. *ChemBioChem* **2007**, *8*, 513. (m) Jordan, J. B.; Shobana, S.; Anderson, O. S.; Hinton, J. F. *Biochemistry* **2006**, *45*, 14012. (n) Pfeifer, J. R.; Reiß, P.; Koert, U. *Angew. Chem., Int. Ed.* **2006**, *45*, 501. (o) Futaki, S.; Zhang, Y.; Kiwada, T.; Nakase, I.; Yagami, T.; Oiki, S.; Sugiura, Y. *Bioorg. Med. Chem.* **2004**, *12*, 1343.

(6) (a) Iwamoto, M.; Shimizu, H.; Muramatsu, I.; Oiki, S. *FEBS Lett.* **2010**, *584*, 3995. (b) Oiki, S.; Muramatsu, I.; Matsunaga, S.; Fusetani, N. *Folia Pharmacol. Jpn.* **1997**, *110* (Suppl. 1), 195.

(7) For total synthesis of polytheonamide B, see: Inoue, M.; Shinohara, N.; Tanabe, S.; Takahashi, T.; Okura, K.; Itoh, H.; Mizoguchi, Y.; Iida, M.; Lee, N.; Matsuoka, S. *Nat. Chem.* **2010**, *2*, 280.

(8) For accounts, see: (a) Inoue, M. *Chem. Rec.* **2011**, *11*, 284. (b) Inoue, M.; Matsuoka, S. *Isr. J. Chem.* **2011**, *51*, 346.

(9) (a) Shinohara, N.; Itoh, H.; Matsuoka, S.; Inoue, M. *ChemMedChem* **2012**, DOI: 10.1002/cmdc.201200142. (b) Matsuoka, S.; Shinohara, N.; Takahashi, T.; Iida, M.; Inoue, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 4879.

(10) For reviews of synthetic channels, see: (a) Matile, S.; Jentzsch, A. V.; Montenegro, J.; Fin, A. *Chem. Soc. Rev.* **2011**, *40*, 2453. (b) Haynes, C. J. E.; Gale, P. A. *Chem. Commun.* **2011**, *47*, 8203. (c) Brotherhood, P. R.; Davis, A. P. *Chem. Soc. Rev.* **2010**, *39*, 3633. (d) Fyles, T. M. *Chem. Soc. Rev.* **2007**, *36*, 335. (e) Davis, J. T.; Okunola, O.; Quesada, R. *Chem. Soc. Rev.* **2010**, *39*, 3843. (f) Gokel, G. W.; Carasel, I. A. *Chem. Soc. Rev.* **2007**, *36*, 378. (g) Ouellet, M.; Otis, F.; Voyer, N.; Auger, M. *Biochim. Biophys. Acta* **2006**, *1758*, 1235. (h) Hector, R. S.; Gin, M. S. *Supramol. Chem.* **2005**, *17*, 129. (i) Koert, U.; Al-Momani, L.; Pfeifer, J. R. *Synthesis* **2004**, 1129. (j) Matile, S.; Som, A.; Sordé, N. *Tetrahedron* **2004**, *60*, 6405. (k) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 988. (l) Koeppe, R. E., II; Andersen, O. S. *Annu. Rev. Biophys. Biomol. Struct.* **1996**, *25*, 231.

(11) For examples, see: (a) Navarro, E.; Fenude, E.; Celda, B. *Biopolymers* **2004**, *73*, 229. (b) Navarro, E.; Fenude, E.; Celda, B.

Biopolymers **2002**, *64*, 198. (c) Lorenzi, G. P.; Gerber, C.; Jäckle, H. *Macromolecules* **1985**, *18*, 154. (d) Lorenzi, G. P.; Jäckle, H; Tomasic, L.; Rizzo, V.; Pedone, C. *J. Am. Chem. Soc.* **1982**, *104*, 1728. (e) Benedetti, E.; Di Blasio, B.; Pedone, C.; Lorenzi, G. P.; Tomasic, L.; Gramlich, V. *Nature* **1979**, *282*, 630.

(12) Structure of a double-stranded dimer of gramicidin A is known. (a) Doyle, D. A.; Wallace, B. A. *J. Mol. Biol.* **1997**, *266*, 963. (b) Langs, D. A.; Smith, G. D.; Courseille, C.; Précigoux, G.; Hospital, M. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5345.

(13) Antiviral peptide antibiotic feglymycin has 13 linear residues of alternating chirality, and adopts a double-stranded antiparallel β-helical dimer. (a) Bunkóczi, G.; Vértesy, L.; Sheldrick, G. M. *Angew. Chem., Int. Ed.* **2005**, *44*, 1340. (b) Vértesy, L.; Aretz, W.; Knauf, M.; Markus, A.; Vogel, M.; Wink, J. *J. Antibiot.* **1999**, *52*, 374. For a total synthesis, see: (c) Dettner, F.; Hänchen, A.; Schols, D.; Toti, L.; Nußer, A.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2009**, *48*, 1856.

(14) Mori, T.; Kokubo, H.; Oiki, S.; Okamoto, Y. *Mol. Simul.* **2011**, *37*, 975.

(15) For reviews on click chemistry, see: (a) Tron, G. C.; Pirali, T.; Billington, R. A.; Canonico, P. L.; Sorba, G.; Genazzani, A. A. *Med. Res. Rev.* **2008**, *28*, 278. (b) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128. (c) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.

(16) Weygand, F.; Steglich, W.; Bjarnason, J. *Chem. Ber.* **1968**, *101*, 3642.

(17) Kaul, R.; Brouillette, Y.; Sajjadi, Z.; Hansford, K. A.; Lubell, W. D. *J. Org. Chem.* **2004**, *69*, 6131.

(18) (a) Kitamura, M.; Shirakawa, S.; Maruoka, K. *Angew. Chem., Int. Ed.* **2005**, *44*, 1549. For reviews, see: (b) Maruoka, K. *Chem. Rec.* **2010**, *10*, 254. (c) Hashimoto, T.; Maruoka, K. *Chem. Rev.* **2007**, *107*, 5656.

(19) Wang, S. *J. Am. Chem. Soc.* **1973**, *95*, 1328.

(20) Chan, W. C.; White, P. D. *Fmoc Solid Phase Peptide Synthesis*; Oxford Univ. Press: New York, 2000.

(21) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

(22) Futaki, S.; Sogawa, K.; Maruyama, J.; Asahara, T.; Niwa, M.; Hojo, H. *Tetrahedron Lett.* **1997**, *38*, 6237.

(23) Residues 26−48 of **3** were synthesized in 1.1% overall yield when the Tmb groups were not introduced to the secondary amides.

(24) (a) Bollhagen, R.; Schmiedberger, M.; Barlos, K.; Grell, E. *J. Chem. Soc., Chem. Commun.* **1994**, 2559. (b) Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 513.

(25) Inverarity, I. A.; Hulme, A. N. *Org. Biomol. Chem.* **2007**, *5*, 636.

(26) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.

(27) (a) Kawakami, T.; Tsuchiya, M.; Nakamura, K.; Aimoto, S. *Tetrahedron Lett.* **2005**, *46*, 5533. (b) Sato, T.; Kawakami, T.; Akaji, K.; Konishi, H.; Mochizuki, K.; Fujiwara, T.; Akutsu, H.; Aimoto, S. *J. Pept. Sci.* **2002**, *8*, 172. (c) Aimoto, S. *Biopolymers* **1999**, *51*, 247. (d) Hojo, H.; Aimoto, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 3055.

(28) Kawakami, T.; Hasegawa, K.; Teruya, K.; Akaji, K.; Horiuchi, M.; Inagaki, F.; Kurihara, Y.; Uesugi, S.; Aimoto, S. *J. Pept. Sci.* **2001**, *7*, 474.

(29) Chelation between Ag^+ and triazoles was reported. (a) Urankar, D.; Pinter, B.; Pevec, A.; De Praft, F.; Turel, I.; Košmrlj, J. *Inorg. Chem.* **2010**, *49*, 4820. (b) Crowley, J. D.; Bandeen, P. H.; Hanton, L. R. *Polyhedron* **2010**, *29*, 70.

(30) (a) Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 4827. (b) Roehm, N. W.; Rodgers, G. H.; Hatfield, S. M.; Glasebrook, A. L. *J. Immunol. Methods* **1991**, *142*, 257.

(31) (a) Bernik, D. L.; Negri, R. M. *J. Colloid Interface Sci.* **1998**, *203*, 97. (b) Waggoner, A. S.; Stryer, L. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 579.

(32) (a) Marsh, D. *Biophys. J.* **2009**, *96*, 2549. (b) Subczynski, W. K.; Wisniewska, A.; Yin, J.-J.; Hyde, J. S.; Kusumi, A. *Biochemistry* **1994**, *33*, 7670.

(33) The CD spectra of polytheonamide B (**1**) and dansylated polytheonamide mimic (**3**) in liposomes were found to be different from that of gramicidin A (see Figure S8 in Supporting Information). These differences are attributable to their side chain hydrogen bond networks, which were not present in the gramicidin structure.

(34) MacDonald, R. C.; MacDonald, R. I.; Menco, B. P. M.; Takeshita, K.; Subbarao, N. K.; Hu, L. *Biochim. Biophys. Acta* **1991**, *1061*, 297.

(35) (a) Sakai, N.; Matile, S. *J. Am. Chem. Soc.* **2003**, *125*, 14348. (b) Weinstein, J. N.; Klausner, R. D.; Innerarity, T.; Ralston, E.; Blumenthal, R. *Biochim. Biophys. Acta* **1981**, *647*, 270.

(36) (a) Otis, F.; Racine-Berthiaume., C.; Voyer, N. *J. Am. Chem. Soc.* **2011**, *133*, 6481. (b) Clement, N. R.; Gould, J. M. *Biochemistry* **1981**, *20*, 1534.

(37) Compounds **1** and **3** were found to be much more efficient ion transporters than gramicidin D under these conditions. See Figure S5 in Supporting Information.

(38) For a review, see: Sakman, B.; Neher, E. *Single-Channel Recording*; Plenum Press: New York, 1995.

(39) (a) Sondermann, M.; George, M.; Fertig, N.; Behrends, J. C. *Biochim. Biophys. Acta* **2006**, *1758*, 545. (b) Fertig, N.; Klau, M.; George, M.; Blick, R. H.; Behrends, J. C. *Appl. Phys. Lett.* **2002**, *81*, 4865. (c) Fertig, N.; Meyer, C.; Blick, R. H.; Trautmann, C.; Behrends, J. C. *Phys. Rev. E.* **2001**, *64*, 040901. (d) Neher, E.; Sakmann, B. *Nature* **1976**, *260*, 799.

(40) See Figure S6 in Supporting Information for the H^+ and Na^+ current traces of **1**.

(41) Typical pK_a value of the dansyl group is approximately 4.0. Koike, T.; Watanabe, T.; Aoki, S.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 12696. Typical pK_a value of the triazole group is 1.2−1.3; thus, we assumed that protonation mainly occurred on the dansyl group. Albert, A.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 19030.

(42) We reported the drastic deactivating effect of the trimethyl ammonium moiety at the N-terminus of polytheonamide B derivative. See reference 9a.

(43) Charged groups attached to the C-terminus of gramicidin A were known to affect its channel behaviors. See for examples: (a) Macrae, M. X.; Blake, S.; Mayer, M.; Yang, J. *J. Am. Chem. Soc.* **2010**, *132*, 1766. (b) Reiß, P.; Al-Momani, L.; Koert, U. *ChemBioChem* **2008**, *9*, 377. (c) Capone, R.; Blake, S.; Restrepo, M. R.; Yang, J.; Mayer, M. *J. Am. Chem. Soc.* **2007**, *129*, 9737. (d) Woolley, G. A.; Zunic, V.; Karanicolas, J.; Jaikaran, A. S. I.; Starostin, A. V. *Biophys. J.* **1997**, *73*, 2465. (e) Woolley, G. A.; Jaikaran, A. S. I.; Zhang, Z.; Peng, S. *J. Am. Chem. Soc.* **1995**, *117*, 4448.