論文の内容の要旨

論文題目：Synthesis, Structural Permutation and Functional Analysis of Polytheonamide Mimic

（ポリセオナミドミミックの合成・構造改変・機能解析）

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Abstract

Introduction

Polytheonamide B (**1**) is a naturally occurring 48-mer peptide, and exhibits extraordinary potent cytotoxicity against P388 mouse leukemia cells (IC_{50} = 0.098 nM).[1] Peptide **1** possesses D,L-alternating amino acid sequence consisting of various non-proteinogenic amino acid components (Figure 1), and is known to fold into a $\beta^{6.3}$-helical conformation in organic solvent (Figure 2a).[2] Interestingly, peptide **1** forms a monovalent-cation selective transmembrane channel in phospholipid bilayers presumably by adopting the $\beta^{6.3}$-helical structure.[3] These unusual features of **1** prompted our group to conduct the total synthesis of **1**[4] and functional analyses of the congeners.[5] The purpose of this research is construction of a more synthetically accessible and more versatile platform to systematically investigate ion channel functions and cytotoxic activities. Herein design, synthesis and functional characterization of dansylated polytheonamide mimic (**2**) were described.[6] Further structure-function analyses of **2** resulted in discovery of a non-channel forming 37-mer cytotoxin **24**.[7]

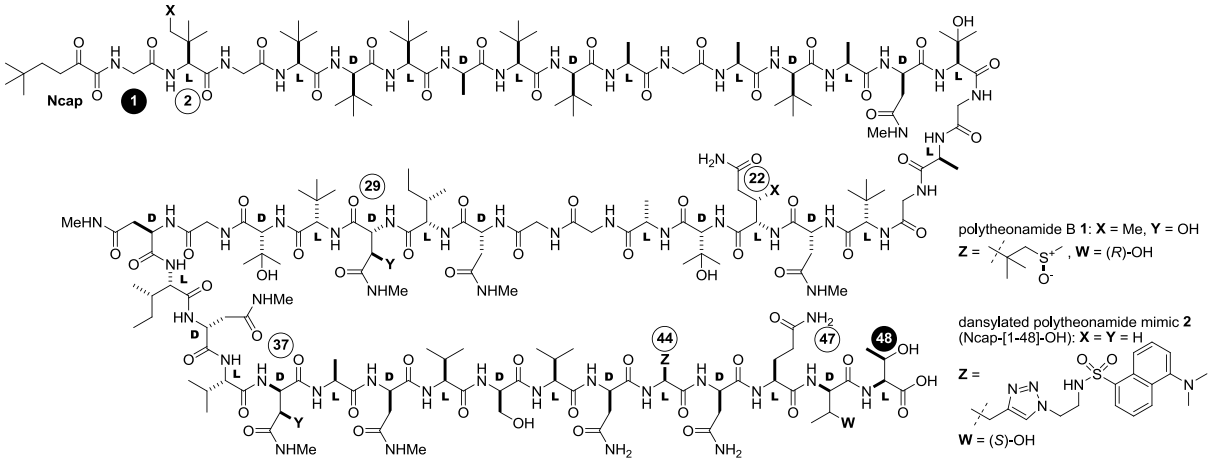


Figure 1. Structures of polytheonamide B (**1**) and dansylated polytheonamide mimic (**2**).

1. Dansylated Polytheonamide Mimic 2 Formed Ion Channels in Phospholipid Bilayers

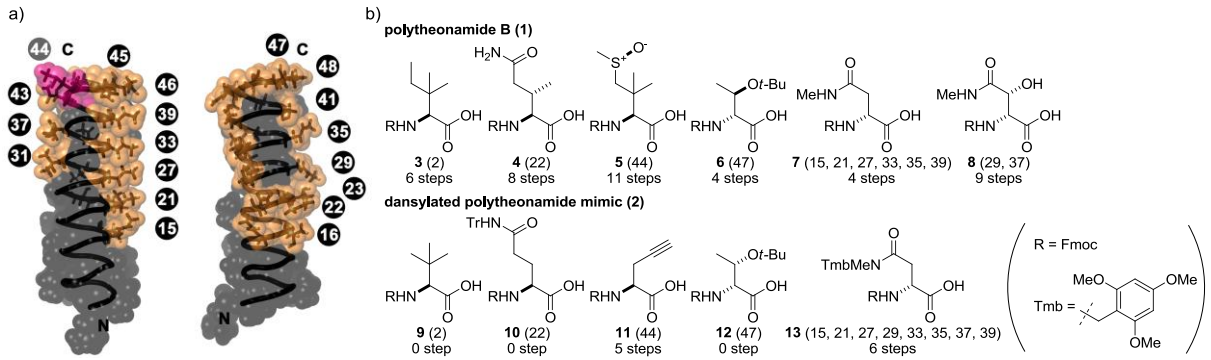
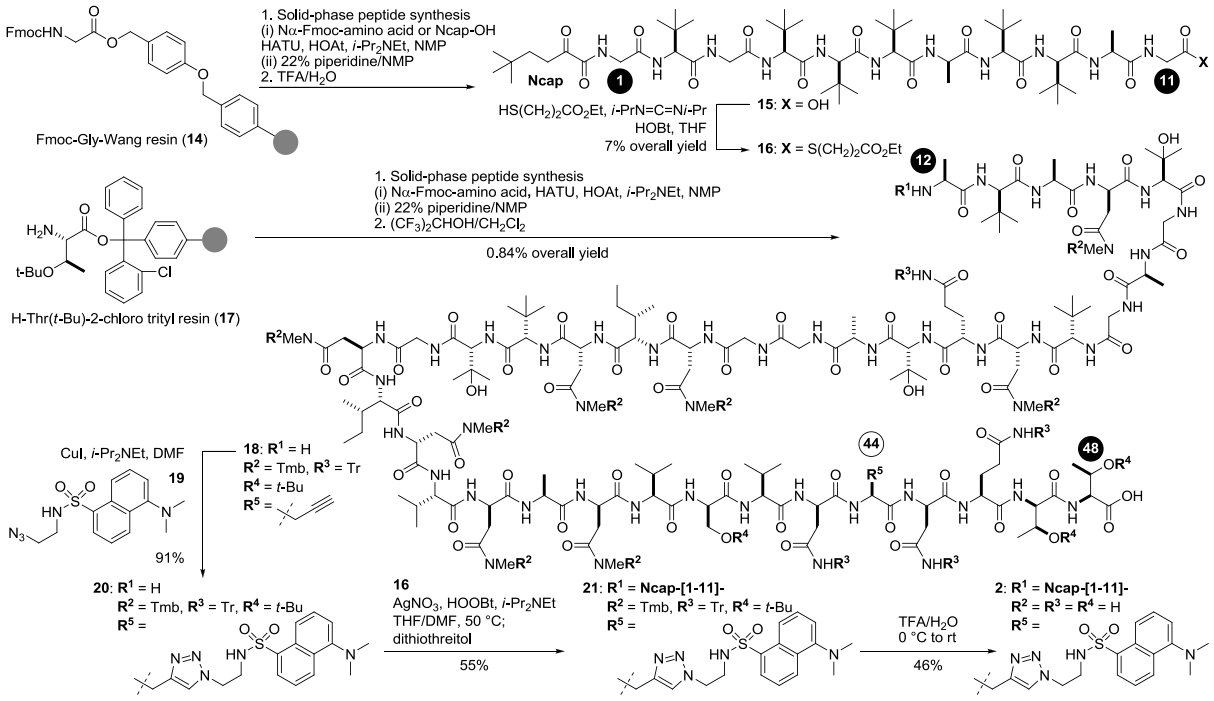


Figure 2. (a) The $\beta^{6.3}$-helix structure of **1** (PDB1D code 2RQO). The structures are rotated by 90-degree around the *y*-axis. The side chains that potentially participate in a hydrogen bonding network along the helix axis are shown with residue numbers. (b) Substituted component monomers in the synthesis of **2**. The numbers indicated in parentheses are residue numbers. The numbers of synthetic steps are shown under the corresponding structures.

Scheme 1. Synthesis of dansylated polytheonamide mimic (**2**).



The total synthesis of **1** has been achieved in 161 total steps. However, the length of the route to **1** limited its supply to perform further studies on molecular functions. To reduce the significant number of synthetic steps, multiple substitutions of the component monomers were planned (Figure 2b). In doing so, the hydrogen bond donors/acceptors in the side chains of **1** were maintained, because the $\beta^{6.3}$-helix of **1** is considered to be stabilized by hydrogen bonds not only of the main chain, but also of the side chains. The side chain of **13** was protected with 2,4,6-trimethoxybenzyl (Tmb) group to prevent interresidue hydrogen bondings of CONHMe groups in solid-phase peptide synthesis (SPPS), because interresidue hydrogen bondings could result in the

formation of nonreactive aggregates in the peptide-resin matrix. In addition, **11** was adopted as a monomer of residue 44, because its terminal alkyne enables facile introductions of various functionalities by 1,3-dipolar cyclization (click reaction) with azide compounds. In this study, polarity-dependent dansyl fluorescent group was incorporated as a reporter of localization of **2** in a phospholipid bilayer.

As expected, the Tmb-protection significantly increased the efficiency of SPPS (Scheme 1). Namely, the 37-mer fragment **18** was synthesized from 2-chloro trityl resin **17**. The introduction of dansyl functionality was attained using **19** with CuI, giving rise to coupling precursor **20**. Optimized Ag^{+}-mediated fragment coupling of amine **20** and thioester **16** afforded **21**. Finally, simultaneous removal of 15 protective groups from **21** was achieved with the treatment of TFA/H_2O. The synthetic strategy to **2** required significantly less synthetic steps in comparison to original **1** (127 steps vs 161 steps) and less technically demanding liquid-phase reactions of the large peptides, demonstrating highly operational superiority of this novel synthetic strategy to construct the 48-mer D,L-alternating sequence.

Interaction between **2** and lipid bilayers was evaluated from solvatochromism of **2** in liposomes. Peptide **2** in liposomes exhibited its Stokes shift as 10564 cm^{-1}. The value was comparable to that of MeOH (10130 cm^{-1}), indicating that C-terminus of **2** is considered to be located to the lipid/water interface.

Formation of ion channels of **2** was evaluated by its single channel currents. In neutral solution (pH 7.4), Na^{+} current of **2** was comparable to that of **1** (Figure 3a). Thus, it was proved **2** emulated the channel function of **1**. Intriguingly, H^{+} current of **2** in acidic solution (pH 1) was remarkably lower than that of **1** (Figure 3b). Since the dansyl group would be located in lipid/water interface, the difference of conductance values can be attributed to protonation of the dansyl group. Namely, the cationic group near the entrance of channel pore is considered to suppress cation flux by its binding to the entrance of the pore or by inducing a repulsive electrostatic effect.[5] These results suggested a possibility of control of channel functions through pH modulation.

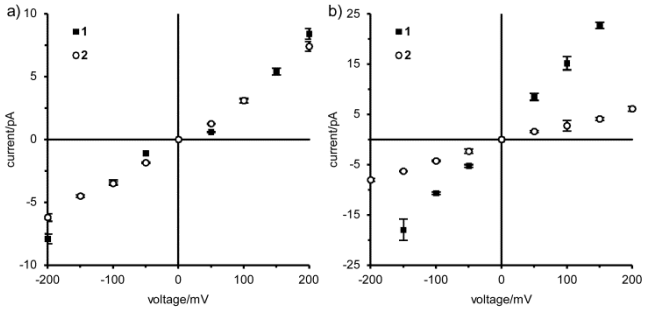


Figure 3. Current-voltage curves of **1** and **2**. Currents show cation flows through DPhPC planer bilayers in (a) 1 M NaCl (pH 7.4) or (b) 0.1 M HCl (pH 1) containing channels formed by **1** and **2**. DPhPC = diphytanoylphosphatidyl choline.

2. Truncated Analog 24 Behaved as a More Potent Cytotoxin than 2 and Exhibited No Ion Transport Activity

In characterization of mimic **2**, IC_{50} value of P388 growth inhibitory activity of **2** was found to be 12 nM (Table 1). This value was still low as a cytotoxic agent, though its cytotoxicity was 100-fold lower than that of **1**. The author next planned to perform comprehensive structure-activity relationship study of the substructures of **2**.

Syntheses and cytotoxicity assay of the 13 truncated analogs **22**–**34** revealed that analog **24** specifically exhibited a more potent cytotoxicity (IC_{50} = 3.7 nM) than that of **2**. Surprisingly, truncated **24** exhibited no ion transportation in liposomes (Figure 4) and in P388 cell membranes. These results suggested a possibility that analog **24** exerted its toxicity through a mode of action distinct from those of **1** and **2**.

Table 1. Cytotoxic activities of synthesized peptides against P388 mouse leukemia cells

compound	IC_{50} (nM)
1: polytheonamide B	0.098
2: Ncap-[1–48]-OH	12
22: H-[10–48]-OH	>420
23: H-[11–48]-OH	>420
24: H-[12–48]-OH	3.7
25: H-[13–48]-OH	81
26: H-[14–48]-OH	100
27: H-[15–48]-OH	>420
28: H-[16–48]-OH	140
29: H-[17–48]-OH	>450
30: H-[18–48]-OH	190
31: H-[19–48]-OH	>410
32: H-[20–48]-OH	390
33: H-[21–48]-OH	>250
34: H-[22–48]-OH	>420

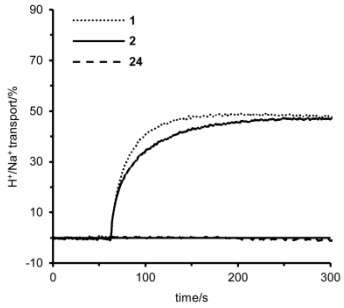


Figure 4. Time-course of H^+/Na^+ exchange across lipid bilayers of liposomes caused by **1**, **2** and **24**. Peptides were added at 60 s.

Conclusion

This work demonstrated that the newly designed synthetic strategy was highly effective to construct the 48-mer D,L-alternating sequence of **2**. The various functional characterizations demonstrated that rationally designed peptide **2** successfully mimicked the channel function of **1**. In addition, truncated **24** was found to have potent cytotoxicity and showed the different behaviors from the parent **1** and **2**. Overall, the newly designed artificial sequence has a potential to be a novel structural platform for designing new peptide ion channels and unique cytotoxic compounds with desired activities.

References

(1) Hamada, T.; Matsunaga, S.; Yano, G.; Fusetani, N. *J. Am. Chem. Soc.* **2005**, *127*, 110.

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

(3) Iwamoto, M.; Shimizu, H.; Muramatsu, I.; Oiki, S. *FEBS Lett.* **2010**, *584*, 3995.

(4) 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.

(5) Shinohara, N.; Itoh, H.; Matsuoka, S.; Inoue, M. *ChemMedChem* **2012**, *7*, 1770.

(6) Itoh, H.; Matsuoka, S.; Kreir, M.; Inoue, M. *J. Am. Chem. Soc.* **2012**, *134*, 14011.

(7) Itoh, H.; Inoue, M. *ACS Med. Chem. Lett.* **2013**, *4*, 52.