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Design and synthesis of an artificial ladder-shaped polyether that interacts with glycophorin A

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Abstract—Ladder-shaped polyether (LSP) compounds, such as brevetoxins and ciguatoxins, are thought to interact with transmembrane (TM) proteins. As a model LSP compound, we designed and synthesized an artificial tetracyclic ether (**1**) and evaluated its interaction with glycophorin A (GpA), a membrane protein known to dimerize or oligomerize between membrane-integral α-helical domains. Model compound **1** was found to induce the dissociation of oligomeric GpA in a similar manner to natural LSPs when examined by SDS–PAGE. The results suggest that even an artificial tetracyclic ether possesses the ability to interact with TM proteins, presumably through the intermolecular hydrogen bonds (C_α–H···O) with the GXXXG motif.

Ladder-shaped polyether (LSP) compounds,[1] including brevetoxin B,[2] ciguatoxin,[3] yessotoxin,[4] and gymnocin-A[5] (Fig. 1), which are unique products of Dinophyceae and Haptophyceae, are composed of continuous *trans*-fused cyclic ethers and are known to possess potent toxicity. Although more than 50 naturally occurring LSPs have been identified, there have been few molecular mode-of-action studies, chiefly due to the short supply of materials. Brevetoxins and ciguatoxins are unusual in that their molecular target has been identified;[6] the toxins share a common binding site on an α subunit of voltage-sensitive sodium channels (VSSCs), with very high affinity (their dissociation constants are in the nanomolar–subnanomolar range). Because VSSCs are comprised of 24 transmembrane (TM) helices, membrane-integral α-helices are considered to be the interacting motif of LSPs. Although other LSPs (e.g., gambierol[7] and gambieric acid A[8]) show weak interaction with VSSCs,[9] they are thought to bind to different molecular targets upon exerting their toxicity. When a polyether matches the binding part of a membrane protein, its binding affinity is greatly enhanced, probably due to an arrangement of polyether oxygen atoms as hydrogen bond acceptors,[9] because the distance between the neighboring skeletal oxygen atoms on the same side of the LSPs is consistent with the helix pitch (ca. 5 Å).[10]

Recently, we developed an assay system for evaluating the interaction between LSPs and membrane-integral α-helices using glycophorin A (GpA), a heavily glycosylated TM protein occurring in erythrocyte membrane which is known to form dimers and oligomers.[11] The activities of LSPs were evaluated on the basis of dissociation of the GpA oligomers and dimers (Fig. 2A), which was detected by SDS–PAGE analysis. Brevetoxin B and yessotoxin were found to induce dissociation of oligomeric GpA into the corresponding dimers or monomers. The structure of the TM region of dimeric GpA in both aqueous detergent micelles and lipid bilayers has been determined by NMR, as illustrated in Figure 2B.[12] Weak hydrogen bonds between the C_α–H of glycine and a carbonyl oxygen (C_α–H···O)[13] were suggested to play an important role in stabilizing the dimeric structure (G79-I76 and G83-V80), while the residual side chains synergistically contribute to stabilization of the complex by van der Waals contact. Recently, the GXXXG sequence motif (the so-called glycine zipper)[14] has commonly been found to mediate TM helix oligomerization. We postulate that the GXXXG sequence may be a candidate for an interacting motif with LSPs, whose oxygen array possibly acts as a series of HB acceptors. According to this hypothesis, a tetracyclic LSP should possess enough length to interact with the GXXXG motif to induce dissociation of the oligomeric

Keywords: Ladder-shaped polyether; Artificial ladder-shaped polyether; Transmembrane protein; Glycophorin A; GXXXG motif.
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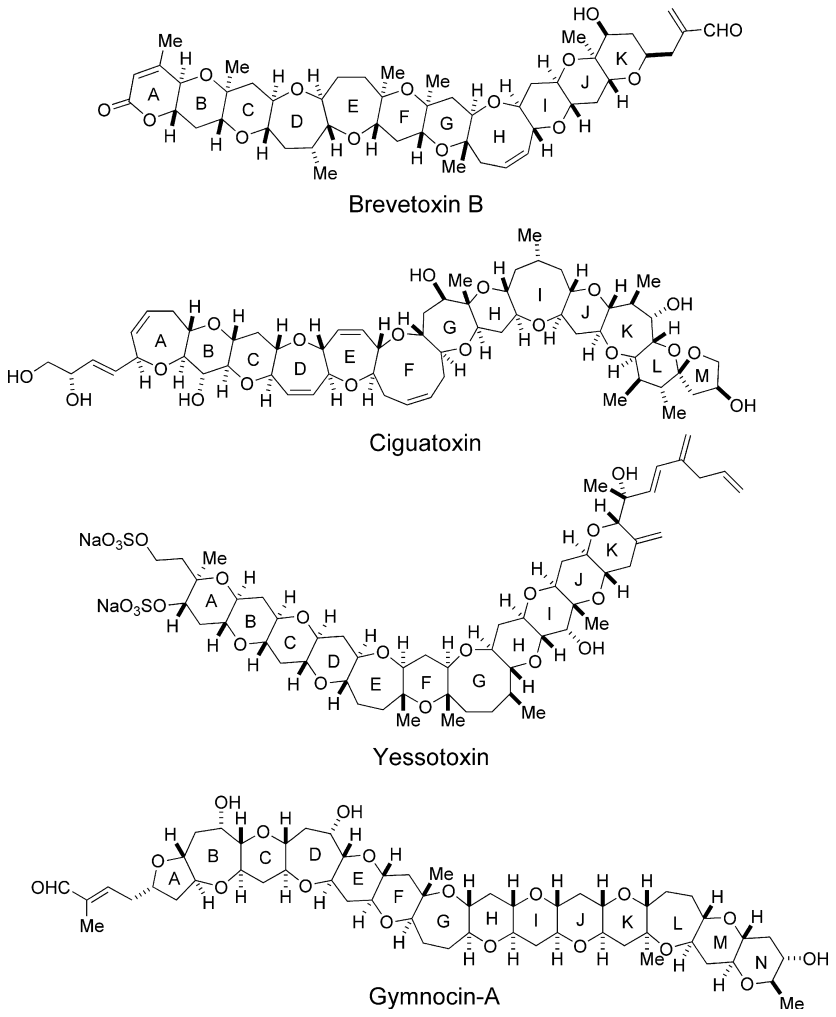


Figure 1. Structures of brevetoxin B, ciguatoxin, yessotoxin, and gymnocin-A.

GpA (Fig. 2C). Therefore, we designed an artificial ladder-shaped polyether (ALP) **1**,[10,15,16] taking account of the following points: (i) a *trans*-fused 6/6/7/6 tetracyclic ether system mimics the partial framework of yessotoxin, gambierol, and gymnocin-A; (ii) the number of ether rings is consistent with that of hemibrevetoxin B, which is the shortest natural LSP; (iii) one of the terminal sides is functionalized with hydroxy groups to increase water solubility, and the other side with benzyl ethers to retain sufficient lipophilicity to access the hydrophobic TM region; (iv) compound **1** could be synthesized expeditiously from fragments **2** and **3** in a convergent manner (Scheme 1). Herein we report the synthesis of ALP **1**, and evaluation by SDS–PAGE of its interaction with GpA based on the dissociation of GpA dimers and oligomers.

Synthesis of **1** commenced with the preparation of fragments **2** and **3** from known tetrahydropyran derivatives **5** and **7** (Scheme 2).[16,17] Oxidation of the primary alcohol **5** followed by treatment of the resulting aldehyde with PhSSPh and *n*-Bu_3P gave dithioacetal **6**, and removal of the TBS group of **6** afforded the secondary alcohol **2**. For carboxylic acid **3**, reductive cleavage of the *p*-methoxybenzylidene acetal **7** proceeded regioselectively to give primary alcohol **8**, which was converted to corresponding iodide **9**. Side-chain elongation of **9** was achieved by means of alkylation with lithium enolate prepared from *t*-butyl acetate, followed by saponification of the resulting ester to afford **3**.

We then moved on to coupling of the fragments and synthesis of the 6/6/7/6 tetracyclic ether **1** as depicted in Scheme 3. Condensation of alcohol **2** with carboxylic acid **3** by treating with EDC·HCl in the presence of DMAP and CSA[18] afforded the ester **10**. The *p*-methoxybenzylidene acetal was converted to di(*t*-butyl)silylene to yield **11** at 86% for three steps. The next task was intramolecular carbonyl olefination mediated by a low-valent titanium complex (Takeda reaction),[19,20] one of the critical points in the present synthesis. Dithioacetal **11** was treated with $Cp_2Ti[P(OEt)_3]_2$ to yield dihydropyran **12**, which was immediately subjected to a hydroboration-oxidation sequence to afford a separable mixture of the desired alcohol **13a** (28%) and diastereomer **13b** (16%). Oxidation of the alcohols **13a** and **13b** furnished ketone **14a** (93%) and **14b** (65%), respectively, and the undesired **14b** was converted to **14a** by treating with DBU at 50 °C. Removal of the PMB group of **14a** using DDQ gave a hydroxy-ketone, which was

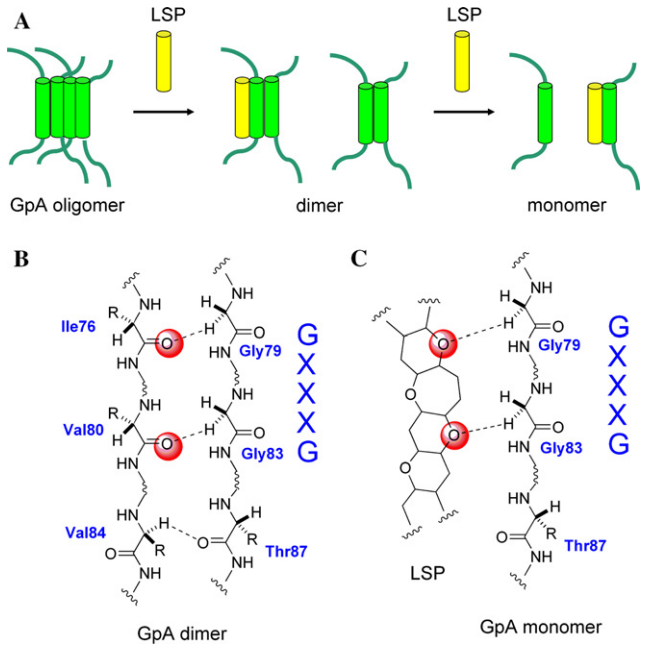
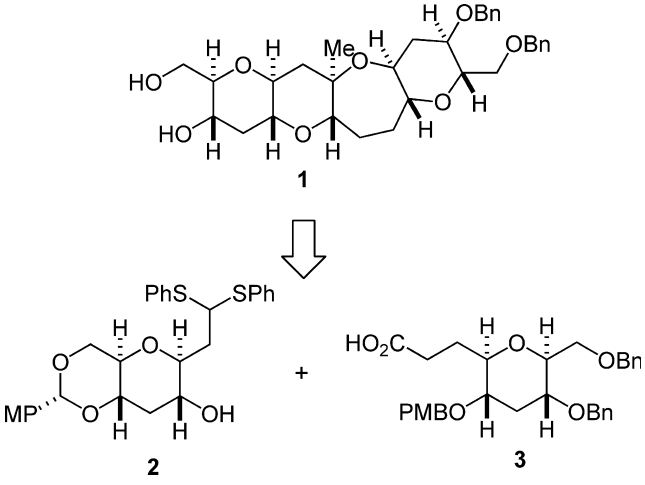
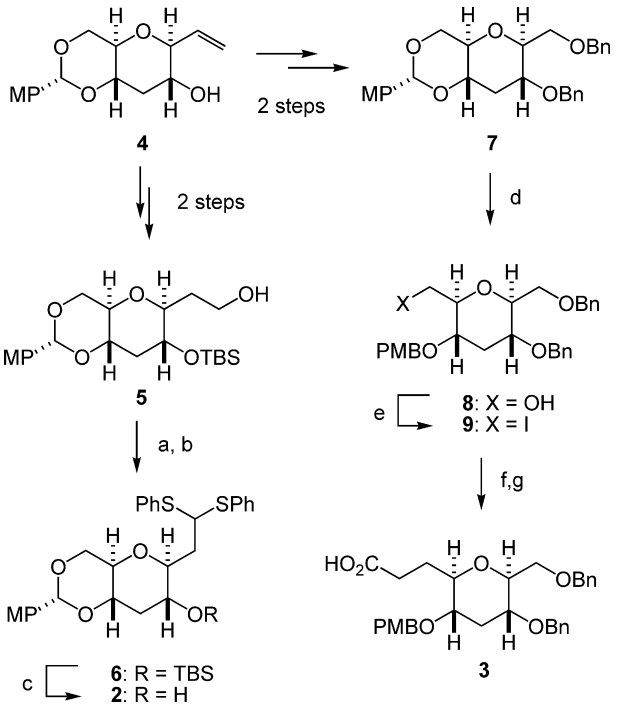


Figure 2. Hypothetical model of LSP-GpA interaction. (A) Illustrative pathway of dissociation of GpA oligomers to dimers or monomers. (B) Schematic drawing of the partial structure of GpA dimer reported by Smith et al. (C) Hypothetical model of complexation of a GpA monomer with a LSP.



Scheme 1. Synthesis plan for the artificial ladder-shaped polyether **1**.

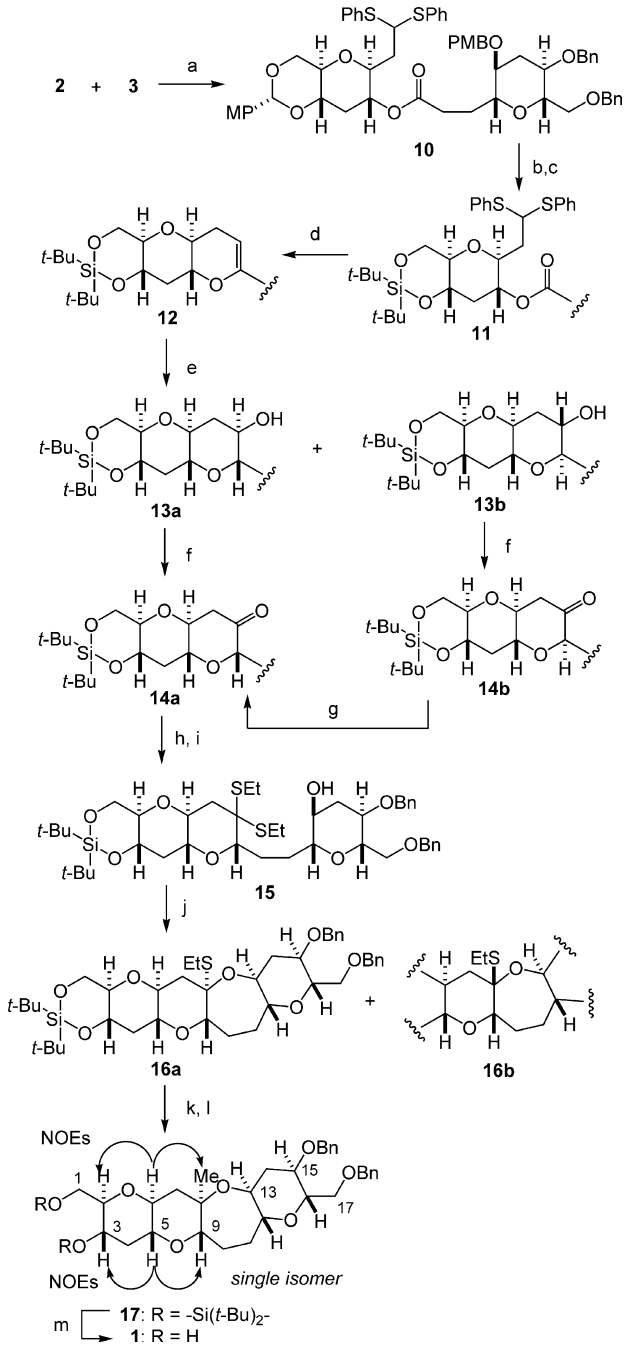


Scheme 2. Reagents and conditions: (a) Dess-Martin periodinane, CH_2Cl_2, rt, quant.; (b) PhSSPh, *n*-Bu_3P, neat, rt; (c) TBAF, THF, rt, 74% (two steps); (d) DIBAL, CH_2Cl_2, −78 to −10 °C, 80%; (e) I_2, PPh_3, imidazole, THF, rt, 90%; (f) *t*-BuOAc, LDA, THF, HMPA, −73 to −15 °C, 78%; (g) KOH, MeOH, THF, H_2O, reflux, 97%.

treated with TMSSEt in the presence of TMSOTf to afford hydroxy dithioacetal **15** (87%). In contrast to the six-membered ring system,[21] a mixed thioacetal was not obtained under these reaction conditions. The formation of the seven-membered ring was then examined by means of Nicolaou's method.[22] Dithioacetal **15** was treated with $AgClO_4$ in the presence of silica gel in nitromethane at room temperature to afford mixed thioacetal **16a** and epimer**16b** in a 1:1.5 ratio (42%); these were separable by silica gel chromatography. The final methylation leading to tetracyclic ether **1** was performed using **16a**. Oxidation of mixed thioacetal **16a** with *m*CPBA toward sulfone, followed by treatment with Me_3Al, afforded *trans*/*syn*-fused **17** as a single isomer (52%). Finally, removal of the silyl group of **17** with TBAF furnished the tetracyclic ether **1**[23] (69%), whose stereochemistry was unambiguously determined by NOE experiments.

We then turned our attention to evaluation of the interaction of **1** with GpA based on dissociating activity by SDS–PAGE (Fig. 3).[11] As shown in lane 1, intact GpA exists as oligomers in various aggregated states, with a significant amount of dimers. When GpA was treated with desulfated yessotoxin (dsYTX, lanes 2–4) the oligomers dissociated dose-dependently to form dimers, and a considerable amount of monomers were also formed (lanes 3 and 4). Meanwhile, treatment of GpA with **1** induced the dissociation of oligomers, as expected (lanes 6 and 7), and the dissociation activity of **1** was comparable to that of dsYTX. It is noteworthy that no significant interaction was observed for monocyclic ether **18**[24] (Fig. 4) possessing the same functional groups as **1** (lanes 8 and 9). As already reported, other artificial polyether compounds, such as polyethylene glycol (lanes 10 and 11) and 18-crown-6, did not show the dissociation activity.[11] These results strongly suggest that the ladder-shaped skeleton, in which the interatomic distances of the neighboring two oxygen atoms on the same side are fixed at a similar distance to the helix pitch, plays an important role in the interaction with TM proteins. These results may be a clue to understanding the mode-of-action of natural LSP toxins.

In conclusion, an artificial ladder-shaped polyether (**1**) was designed with the aim of evaluating its interaction with transmembrane protein glycophorin A, and



Scheme 3. Reagents and conditions: (a) EDC·HCl, DMAP, CSA, CH_2Cl_2, reflux; (b) TsOH, H_2O, MeOH, rt; (c) t-$Bu_2Si(OTf)_2$, 2,6-lutidine, DMF, 0 °C, 86% (three steps); (d) $Cp_2Ti[P(OEt)_3]_2$, MS4A, THF, rt; (e) BH_3·THF, 0 °C to rt, then $NaBO_3$, H_2O, THF, reflux (**13a**, 28%; **13b**, 16% for two steps); (f) Dess-Martin periodinane, CH_2Cl_2, rt, 93% (for **13a**–**14a**); TPAP, NMO, MS4A, CH_2Cl_2, rt, 65% (for **13b**–**14b**); (g) DBU, toluene, 50 °C, 47%; (h) DDQ, H_2O, CH_2Cl_2, 0 °C to rt, 63%; (i) TMSSEt, TMSOTf, CH_2Cl_2, −78 to −40 °C, 87%; (j) $AgClO_4$, $NaHCO_3$, SiO_2, MS4A, CH_3NO_2, rt, 90 min, 42% (**16a**:**16b** = 1:1.5, separable); (k) mCPBA, $NaHCO_3$, CH_2Cl_2, rt; (l) Me_3Al, CH_2Cl_2, −50 to −20 °C, 52% (two steps); (m) TBAF, THF, rt, 69%.

synthesized in a convergent manner via intramolecular carbonyl olefination and hydroxydithioacetal cyclization. Based on SDS–PAGE analysis, we demonstrated

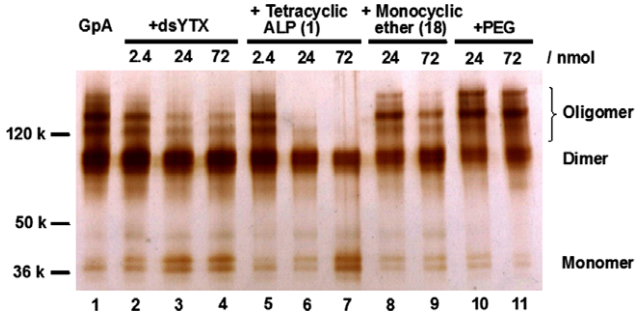


Figure 3. SDS–PAGE of GpA in the presence of natural and artificial polyethers. GpA (2.6 pmol, 98 ng) alone (lane 1); in the presence of 2.4 nmol (lane 2), 24 nmol (lane 3), and 72 nmol (lane 4) of desulfated yessotoxin (dsYTX); in the presence of 2.4 nmol (lane 5), 24 nmol (lane 6), and 72 nmol (lane 7) of ALP **1**; in the presence of 24 nmol (lane 8) and 72 nmol (lane 9) of ALP **18**; in the presence of 24 nmol (lane 10) and 72 nmol (lane 11) of PEG. GpA was visualized by silver staining.

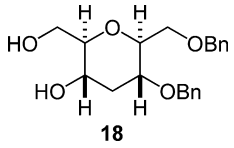


Figure 4. Structure of monocyclic ether **18**.

that even the tetracyclic ALP possesses the ability to induce dissociation of glycophorin A oligomers to form dimers and monomers. In order to elicit the precise mechanism of molecular recognition, synthesis of more ingeniously designed ALPs is in progress in our laboratory.

Acknowledgments

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23. Compound **1**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19–7.31 (10H, m, Ph), 4.58 (1H, d, J = 12.5 Hz, Bn), 4.56 (1H, d, J = 11.0 Hz, Bn), 4.52 (1H, d, J = 12.5 Hz, Bn), 4.35 (1H, d, J = 11.0 Hz, Bn), 3.86 (1H, dd, J = 11.5, 4.0 Hz, H1), 3.76 (1H, dd, J = 11.5, 4.0 Hz, H1), 3.71 (1H, dd, J = 12.0, 1.0 Hz, H17), 3.67 (1H, ddd, J = 9.0, 9.0, 4.5 Hz, H3), 3.57 (1H, dd, J = 12.0, 5.5 Hz, H17), 3.48 (1H, ddd, J = 11.0, 11.0, 5.0 Hz, H13), 3.43 (1H, ddd, J = 12.0, 11.0, 5.0 Hz, H15), 3.30 (1H, m, H12), 3.22 (2H, m, H2, H16), 3.17 (1H, dd, J = 10.5, 4.5 Hz, H9), 3.10 (1H, ddd, J = 12.5, 12.5, 4.0 Hz, H6), 2.96 (1H, ddd, J = 12.5, 12.5, 4.5 Hz, H5), 2.42 (1H, ddd, J = 12.5, 5.0, 5.0 Hz, $H14_{eq}$), 2.37 (1H, ddd, J = 12.0, 4.5, 4.5 Hz, $H4_{eq}$), 2.13 (1H, m, H11), 2.03 (1H, dd, J = 12.5, 4.0 Hz, $H7_{eq}$), 1.83 (1H, m, H10), 1.74 (2H, m, H10, 11), 1.41–1.56 (3H, m, $H4_{ax}$, $H7_{ax}$, $H14_{ax}$), 1.23 (3H, s, Me); ESI-MS m/z 577 ($M+Na^+$).
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