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Design and Synthesis of Ladder-Shaped Tetracyclic, Heptacyclic, and Decacyclic Ethers and Evaluation of the Interaction with Transmembrane Proteins

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Abstract: Ladder-shaped polyether (LSP) toxins represented by brevetoxins and ciguatoxins are thought to bind to transmembrane (TM) proteins. To elucidate the interactions of LSPs with TM proteins, we have synthesized artificial ladder-shaped polyethers (ALPs) containing 6/7/6/6 tetracyclic, 6/7/6/6/7/6/6 heptacyclic, and 6/7/6/6/7/6/6/7/6/6 decacyclic systems, based on the convergent method via α-cyano ethers. The ALPs possessing the simple iterative structure with different numbers of rings would be useful for structure−activity relationship studies on the molecular length, which is supposed to be important when naturally occurring LSPs elicit their toxicity. Two series of ALPs were prepared to evaluate the hydrophilic or hydrophobic effects of the side chains: (i) both sides were functionalized as diols (A series), and (ii) one side remained as diol and the other side was protected as benzyl ethers (B series). To examine the interaction of these ALPs with TM proteins, dissociation of glycophorin A (GpA) dimers into monomers was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The heptacyclic ether (ALP7B) elicited the most potent activity in the presence of 2% SDS buffer, whereas the decacyclic ether (ALP10A) exhibited an intriguing phenomenon to induce precipitation of GpA in a dose-dependent manner, under the low concentration of SDS (0.03%). ALP10A also induced precipitation of integrin $\alpha_1\beta_1$, a TM protein known to form heterodimers in the lipid bilayer membranes. The different activities among the ALPs can be accounted for by the concept of "hydrophobic matching" that is, lengths of the hydrophobic region including the side chains of ALP7B and ALP10A are ca. 25 Å, which match the lengths of the hydrophobic region of α-helical TM proteins, as well as the hydrophobic thickness of lipid bilayer membranes. The concept of the hydrophobic matching would be a clue to understanding the interaction between LSPs and TM proteins, and also a guiding principle to design ALPs possessing potent affinities with TM proteins.

Introduction

Ladder-shaped polyether (LSP) compounds are unique natural products of marine unicellular algae called dinoflagellates (Figure 1).[1] Brevetoxin-B is the first example of LSP, which was isolated from *Karenia brevis* (formerly *Gymnodinium breve*) in association with "red tide", and whose structure was elucidated in 1981 to be an unprecedented undecacyclic ether.[2] More than 50 LSPs have been identified to date including their congeners, which possess the general structural motif of continuous trans/syn-fused ether rings as shown in Figure 1; brevetoxin-A,[3] hemibrevetoxin-B,[4] brevenal,[5] ciguatoxin,[6] CTX3C,[7] gambieric acid-A,[8] gambierol,[9] gymnocin-A,[10] yessotoxin,[11] etc.[12] The structural diversity of LSPs is attributed to various combinations of the number and sizes of cyclic ethers ranging from five- to nine-membered rings, and the order of the ring connection results in considerable skeletal diversity. These LSPs elicit a broad spectrum of biological activities; for example, brevetoxins (LD_{50} > 200 μg/kg, mice, i.p.)[13] and ciguatoxins (LD_{50} = 0.25∼3.6 μg/kg, mice, i.p.)[6b,7,14] are potent neurotoxins, while gymnocin-A exhibits cytotoxicity against P388 mouse leukemia cells (ED_{50} = 1.3 μg/mL).[10] Gambieric acid-A is not a mammalian toxin but an antifungal, which is 2000 times more potent than amphotericin B.[15] Yessotoxin induces apoptosis via a mitochondrial signal transduction pathway.[16] Despite the intriguing biological activities, there have been few mode-of-action studies at the molecular level, chiefly because of the short supply of materials. Brevetoxins and ciguatoxins are unusual in that their molecular target has been

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identified;[17] the toxins share a common binding site on an α subunit of voltage-sensitive sodium channels (VSSCs), with very high affinity as shown by their dissociation constants in the nanomolar-subnanomolar range. Because of the structural similarity, molecular targets of LSPs are considered to be transmembrane (TM) proteins including ion channels or receptors. For mode-of-action studies of LSPs, most of which are hardly obtainable from natural sources, total synthesis of the LSPs, in which remarkable progress has been made in the recent decade,[18] should be crucial. As a matter of fact, by using synthetic specimens,[19,20] it has been revealed that gambierol selectively inhibits the voltage-gated K^+ channels in mouse taste cells, while CTX3C is ineffective against the same channels, although markedly affecting the ion currents through voltage-gated Na^+ channels.[21] Furthermore, gambierol has recently been shown to be a TRPV1 (transient receptor potential vanilloid 1) antagonist.[22]

It is reported that the binding of PbTx-3 (a brevetoxin-B derivative) to VSSCs was competitively inhibited by the addition of not only ciguatoxin ($K_i = 0.15$ nM) but also gambieric acid-A ($K_i = 0.11$ μM) and gambierol ($K_i = 1.4$ μM) while their affinities are $10^4 \sim 10^6$ times lower than that of ciguatoxin.[23] Although it is suggested that there is a significant relationship between the size of the polycyclic region and the inhibitory activity,[23,24] yessotoxin did not inhibit the binding despite the same number of rings as brevetoxin-B (undecacyclic).[23] In addition, it is reported that the K_i value of a small pentacyclic LSP, brevenal,[5] is 1.85 μM, which is comparable to that of gambierol (heptacyclic). Therefore, not only the size of the polycyclic region (number of rings), but also molecular shape and functional groups of the LSP should be taken into consideration upon estimating the affinity of LSPs to VSSCs.

The structural diversity of natural LSPs, differing in length (including the number of rings and side chains), shape (the order of connected ring sizes), and functional groups, has been an obstacle for quantitative structure−activity relationship (SAR) studies. To sort out the size effects of the polycyclic region from other factors on the biological activities, it is necessary to prepare model compounds as molecular probes that are composed of a consistent ring sequence with different length, which are unavailable from natural sources. Recently, syntheses of model compounds mimicking LSPs have been reported by Martin,[25] Oguri,[26] Tachibana,[27] and our groups,[28,29] for the purpose of elucidating the molecular basis for biological

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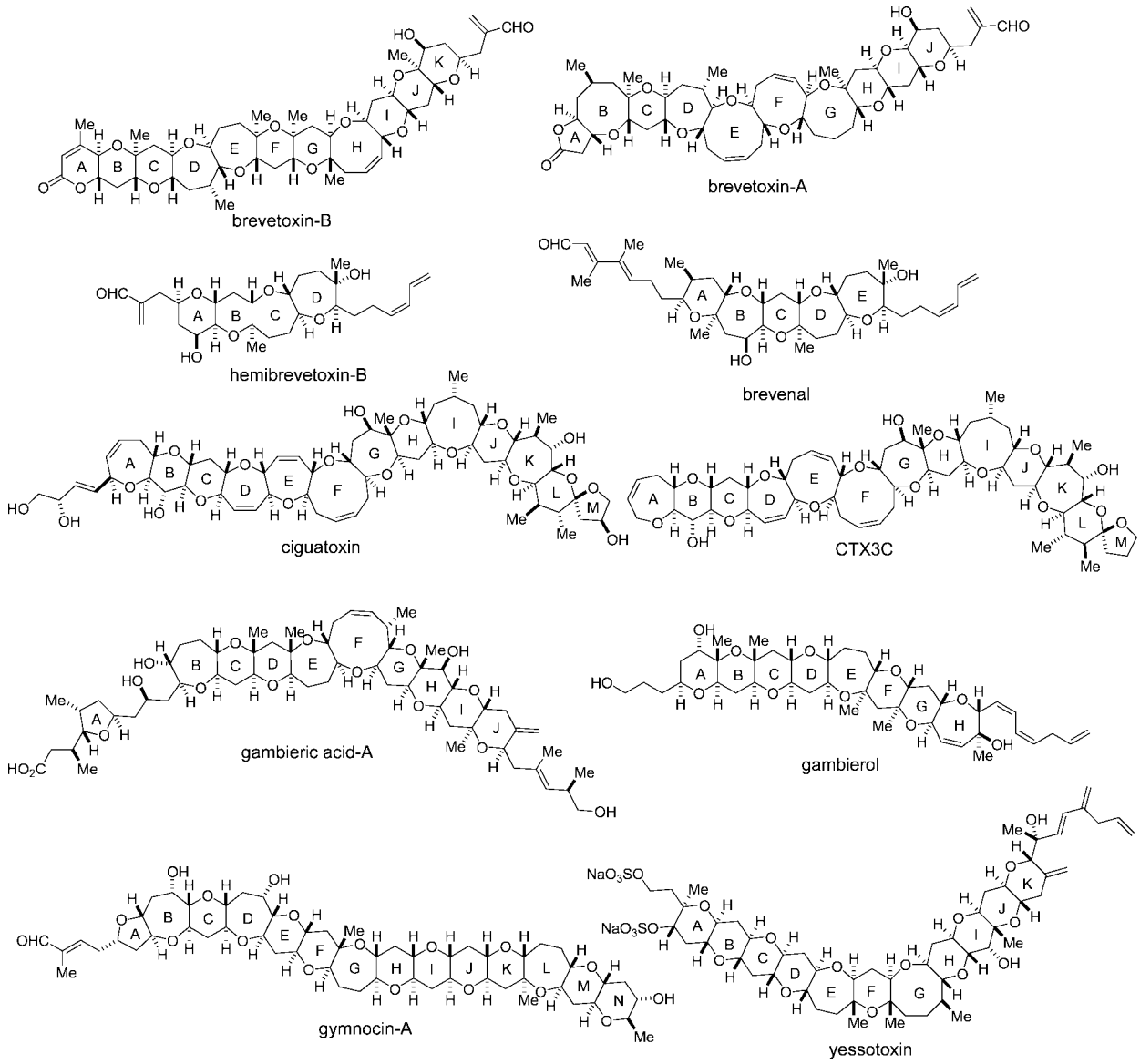

Figure 1. Structures of representative ladder-shaped polyethers from marine origin.

activities. However, the number of rings is limited to the pentacyclic or the heptacyclic system. Herein, we report design and systematic synthesis of artificial ladder-shaped polyethers (ALPs) with tetracyclic, heptacyclic, and decacyclic systems encompassing iterative 6/7/6 tricycles. Studies on the evaluation of the interaction with TM proteins are also reported.

Results

Design and Synthesis of Artificial Ladder-Shaped Polyethers. We designed 6/7/6/6 tetracyclic, 6/7/6/6/7/6/6 heptacyclic, and 6/7/6/6/7/6/6/7/6/6 decacyclic ALPs (**1**∼**3**, Figure 2), which possess a common iterative 6/7/6 ring system with different molecular length, by the following reasons: (i) a trans-fused 6/7/6 ring system mimics the partial framework frequently occurring in natural LSPs such as yessotoxin, gambierol, and gymnocin-A, with placing angular methyl(s) to improve solubility of the ALPs in the lipid membranes; (ii) one of the terminal sides is functionalized as diol to increase water solubility and the other side was equipped with hydroxy groups (termed as ALPA series) or benzyloxy groups (ALPB series) to assess the hydrophilic or hydrophobic effects of the side chains; (iii) these ALPs can be efficiently synthesized in a convergent manner.

Significant advancements in the construction of polycyclic ether systems based on the one-pot strategies have been made by epoxide-opening cascades[30] and two-directional elongation.[31] Recently, we have reported the synthesis of a tetracyclic model compound (Figure 3A) by using Takeda cyclization,[28] and the

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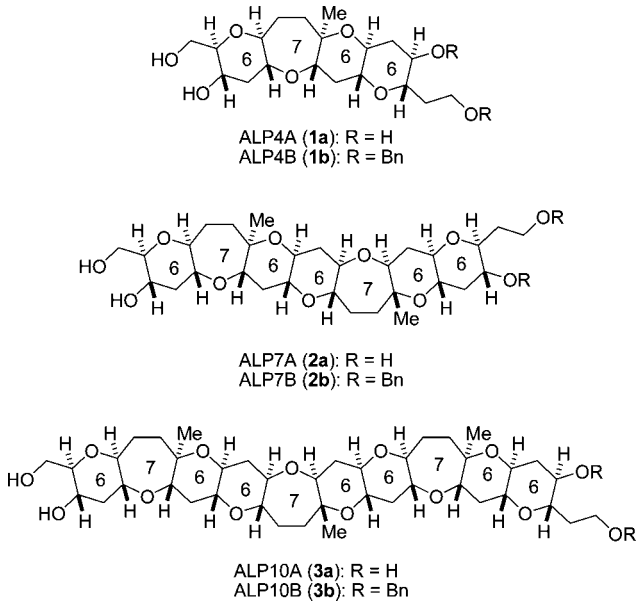


Figure 2. Structures of artificial ladder-shaped polyethers (ALPs).

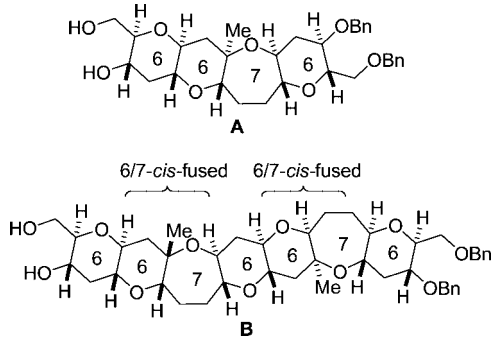
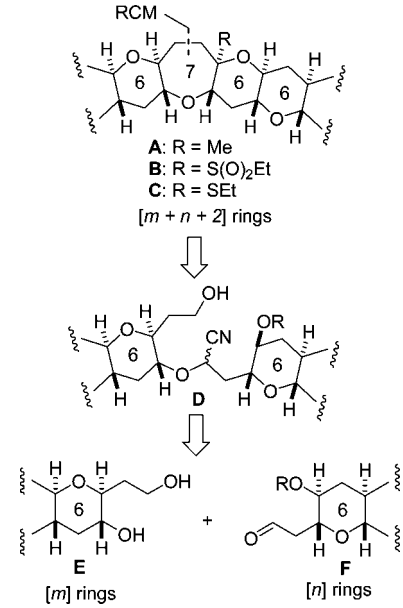


Figure 3. Structures of model compounds.

methodology was applied to a heptacyclic ether on the basis of the double reaction strategy.[29] However, construction of the seven-membered ring and alkylation to introduce angular methyl groups were problematic in terms of yield and stereoselectivity to give 6/7-cis-fused product as a major component (Figure 3B). In this study, we envisaged a convergent strategy[18] via α-cyano ethers (α-cyano ether method) developed in our laboratory[32] for the synthesis of the ALPs (**1**−**3**), which was successfully applied to the convergent synthesis of the CDEF-[33] and FGHI-ring[34] fragments of yessotoxin. As illustrated in Scheme 1, a 6/7/6/6-fused system possessing an angular methyl group (**A**), would be derived from α-cyano ether (**D**) via ring closing metathesis (RCM)[35,36] for the construction of the seven-membered ring, followed by mixed thioacetal (**C**) formation and alkylation of the corresponding sulfone (**B**). The key intermediate (**D**) could be retrosynthetically dissected into diol (**E**) and aldehyde (**F**). In this strategy, two rings are constructed through the coupling of fragments, therefore, [$m + n + 2$] ring

Scheme 1. Synthetic Strategy Based on the Convergent Method via α-Cyano Ethers



system being obtained from [m] and [n] ring fragments (m, n: number of rings of the fragments). However, it was uncertain whether the α-cyano ether method was applicable for synthesizing a large molecule such as a decacyclic system.

Synthesis of the 6/7/6/6 tetracyclic ALP (**1**) based on the α-cyano ether method[32] is depicted in Scheme 2. Tetrahydropyran derivative **4b**[34,37] was chosen as a pivotal intermediate for synthesizing the ALPs. The dibenzylether **4b** and tetraol **4a** derived from **4b** by means of hydrogenolysis were used as monocyclic model compounds termed as ALP1B (**4b**) and ALP1A (**4a**), respectively, to compare with the ALPs (**1**−**3**). Condensation of diol **5**[32] and aldehyde **6**[34] derived from **4b** according to the reported procedure, respectively, proceeded smoothly by the action of $Sc(OTf)_3$ to give seven-membered cyclic acetal in 95% yield. Regioselective opening of the acetal was achieved by treatment with TMSCN in the presence of $Sc(OTf)_3$ at room temperature for one hour to form α-cyano ether **7** (53%) with the recovery of the starting material (44%), which was recycled three times to provide **7** in 82% total yield. Although prolonged reaction time of the acetal cleavage resulted in removal of the PMB group, it would be overcome by using 2-naphthylmethyl (NAP) group instead of PMB.[34] Sequential conversion of primary alcohol **7** into terminal olefin **8** via 2-nitrophenyl selenide was unsuccessful under the standard conditions.[38] After considerable experimentation, we found that the addition of MS4A is requisite for conversion of **7** into the

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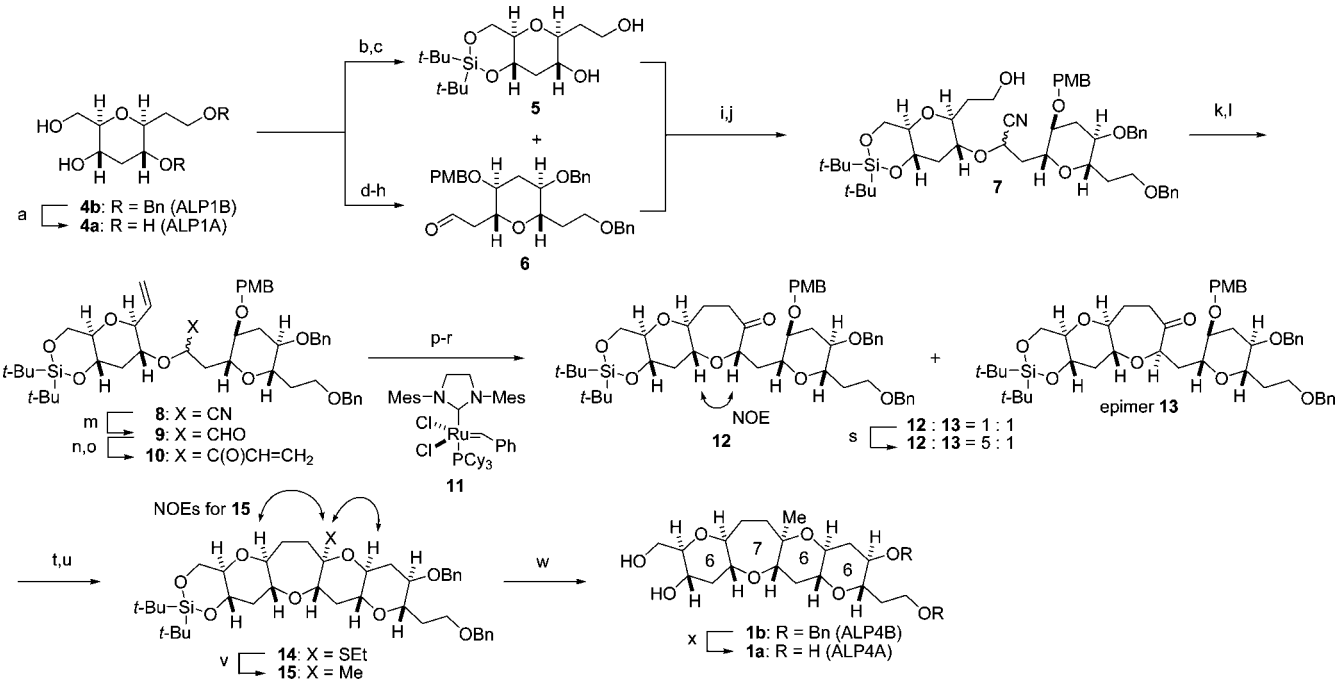
Scheme 2. Synthesis of the Tetracyclic ALPs (**1**)[a]



[a] Reagents and conditions: (a) H_2, $Pd(OH)_2$/C, THF, room temperature (rt), 3.5 d, 92%; (b) (*t*-Bu)$_2$Si(OTf)$_2$, 2,6-lutidine, DMF, 0 °C, 20 min; (c) H_2, $Pd(OH)_2$/C, THF, rt, 18 h, 97% (two steps); (d) *p*-$MeOC_6H_4CH(OMe)_2$, CSA, CH_2Cl_2, rt, 4.5 h, 91%; (e) DIBALH, CH_2Cl_2, −78 to −11 °C, 2 h; (f) MsCl, Et_3N, CH_2Cl_2, 0 °C, 20 min; (g) NaCN, 18-crown-6, DMF, 80 °C, 4 h, 81% (three steps); (h) DIBALH, CH_2Cl_2, −67 to −25 °C, 1.5 h, 86%; (i) $Sc(OTf)_3$, benzene, rt, 40 min, 95%; (j) TMSCN, $Sc(OTf)_3$, CH_2Cl_2, rt, 1 h, 82% (after three cycles); (k) *o*-NO_2−C_6H_4SeCN, *n*-Bu_3P, MS4A, THF, rt, 1 h; (l) *m*CPBA, 1,2-dichloroethane, rt, 15 min, then $NaHCO_3$, 60 °C, 25 min, 74% (two steps); (m) DIBALH, toluene, −78 °C, 1 h, 71%; (n) tetravinyltin, MeLi, THF, −78 to −62 °C, 12 min, 90%; (o) Dess−Martin periodinane, CH_2Cl_2, rt, 1 h, 97%; (p) Grubbs cat. (2nd generation) **11**, toluene, reflux, 15 min, 95%; (q) H_2, PtO_2, AcOEt, rt, 3.5 h; (r) Dess-Martin periodinane, CH_2Cl_2, rt, 2 h, 96% (two steps); (s) DBU, toluene, reflux, 39 h, 93%; (t) DDQ, H_2O, CH_2Cl_2, rt, 3.5 h, 68%, (epimer 11%); (u) EtSH, $Zn(OTf)_2$, CH_2Cl_2, 0 to 14 °C, 2.5 h, 86%; (v) *m*CPBA, CH_2Cl_2, rt, 30 min, then Me_3Al, −40 to −20 °C, 1 h, 91%; (w) TBAF, THF, rt, 24 h, 85%; (x) H_2, $Pd(OH)_2$/C, THF, rt, 10 h, quant.

corresponding selenide, and *m*CPBA was superior to hydrogen peroxide in the oxidation of the selenide giving selenoxide. Thus, olefin **8** was successfully obtained in 74% yield for two steps. Reduction of the nitrile **8** with DIBALH gave aldehyde **9**, which was converted into enone **10** by treatment with vinyllithium followed by Dess−Martin oxidation[39] of the resulting allylic alcohol (62%, three steps). RCM of the diene **10** by the action of second generation Grubbs catalyst **11** (3.5 mol %)[40] proceeded smoothly in toluene at reflux to afford the seven-membered cyclic enone in 95% yield. Hydrogenation of the enone using PtO_2 gave a mixture of saturated ketones with concomitant formation of alcohols by overreaction, which was readily oxidized to give ketone **12** and **13** as a mixture of diasteomers in a 1:1 ratio (96%, two steps). Conversion of the undesired epimer **13** into **12** was achieved by treatment with DBU in toluene at 110 °C (**12**:**13** = 5:1), and the structure of **12** was confirmed by NOE experiments. Removal of the PMB group using DDQ (at this stage, the minor epimer was removed by silica gel column chromatography), followed by treatment with EtSH in the presence of $Zn(OTf)_2$[41] yielded cyclic mixed thioacetal **14** (58%, two steps). Stereoselective introduction of an angular methyl group[42] was successfully achieved through oxidation of mixed thioacetal **14** with *m*CPBA toward sulfone, and one-pot treatment of the reaction mixture with Me_3Al to afford **15** (91%) as a single isomer. The configuration of the angular methyl group was unambiguously determined by NOE experiments. Removal of the silyl group of **15** with TBAF yielded **1b** (ALP4B) in 85% yield. Thus, 6/7/6/6 tetracyclic ALP4B was synthesized from monocyclic building blocks (**5** and **6**) in 14% overall yield for 14 steps (87% average yield). Hydrogenolysis of the benzyl ether **1b** afforded tetraol **1a** (ALP4A) quantitatively.

The heptacyclic ALP **2** was synthesized in an analogous sequence as ALP **1** based on the α-cyano ether method, starting from diol **16** prepared from tetracyclic ether **15** (Scheme 3). Coupling of the diol **16** with aldehyde **6** by means of acetal formation and regioselective cleavage to provide α-cyano ether **17**, followed by construction of seven-membered ring through RCM and epimerization afforded ketone **18**. Subsequent six-membered ring formation was successfully achieved in a stereoselective manner (confirmed by NOE experiments of **19**) to afford 6/7/6/6/7/6/6 heptacyclic **2b** (ALP7B). The overall yield from **16** and **6** was 6.6%, and the average yield was calculated to be 82% for 14 steps. Removal of the benzyl groups of **2b** gave tetraol **2a** (ALP7A).

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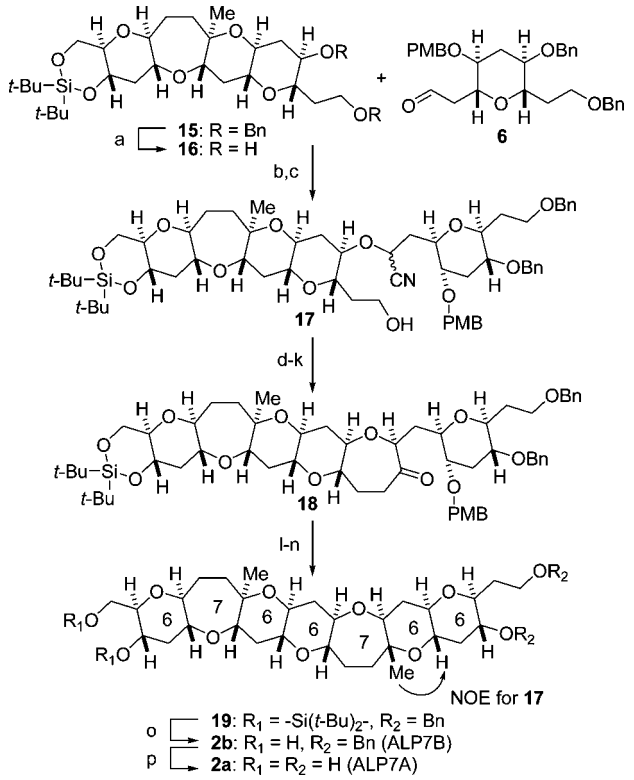
Scheme 3. Synthesis of the Heptacyclic ALPs (**2**)[a]



[a] Reagents and conditions: (a) H_2, $Pd(OH)_2$/C, THF, rt, 6 h, quant.; (b) $Sc(OTf)_3$, benzene, rt, 1 h, 79%; (c) TMSCN, $Sc(OTf)_3$, CH_2Cl_2, rt, 80 min, 67%; (d) *o*-NO_2−C_6H_4SeCN, *n*-Bu_3P, MS4A, THF, rt, 40 min; (e) *m*CPBA, 1,2-dichloroethane, rt, 30 min, then $NaHCO_3$, 60 °C, 30 min, 71% (two steps); (f) DIBALH, CH_2Cl_2, −78 °C, 50 min, 63%; (g) tetravinyltin, MeLi, THF, −78 to −67 °C, 20 min, 91%; (h) Dess−Martin periodinane, CH_2Cl_2, rt, 50 min, 92%; (i) Grubbs cat. (2nd generation) **11**, toluene, reflux, 20 min, 95%; (j) H_2, PtO_2, AcOEt, rt, 2.5 h, then Dess−Martin periodinane, CH_2Cl_2, rt, 75 min, 94%; (k) DBU, toluene, reflux, 64 h, 82%; (l) DDQ, H_2O, CH_2Cl_2, rt, 50 min, 59%; (m) EtSH, $Zn(OTf)_2$, CH_2Cl_2, 0 °C to rt, 3 h, 79%; (n) *m*CPBA, CH_2Cl_2, 0 °C, 30 min, then Me_3Al, −55 to −32 °C, 40 min, quant.; (o) HF·pyridine, THF, 0 to 10 °C, 3 h, quant.; (p) H_2, $Pd(OH)_2$/C, THF, rt, 10 h, quant.

The remaining task is synthesis of the decacyclic ALP (**3**), the most challenging molecule in the present study (Scheme 4). In an analogous sequence as the synthesis of ALPs **1** and **2**, coupling of diol **16** and aldehyde **20** (prepared from **1a** by the identical procedure from **4b** to **6**, Scheme 2) provided α-cyano ether **21**, and conversion to decacyclic ether **23** via ketone **22** proceeded smoothly irrespective of the size of the synthetic intermediates. Although it was not an easy task to determine the configuration of the newly formed angular methyl group of ALP **3** because of the overlapped NMR signals due to its iterative structure, the 920 MHz ^{1}H NMR was of great use for structure determination, which enabled the NOE experiments by selective irradiation of the individual angular methyl groups of **23** (Supporting Information). Removal of the silyl group of **23** afforded 6/7/6/6/7/6/6/7/6/6 decacyclic **3b** (ALP10B). The average yield from **16** and **3b** for 14 steps was 83% (6.8% total yield), which is comparable to that of the heptacyclic ALP. Finally, hydrogenolysis of benzyl ether **3b** gave tetraol **3a** (ALP10A).

Thus, systematic synthesis of ALPs composed of the tetracyclic, heptacyclic, and decacyclic systems has been successfully achieved in a convergent manner. The α-cyano ether method proved to be a powerful strategy, not only for coupling of small fragments, but also for large segments giving decacyclic ether system which is the largest ALP to date possessing iterative structure. These ALPs with different number of rings comprising a 6/7/6 iterative sequence are useful molecular probes for SAR studies on the interaction with TM proteins and biological activities.

Evaluation of the Interaction between ALPs and TM Proteins. To evaluate the interaction of the ALPs with TM proteins, glycophorin A (GpA), a heavily glycosylated TM protein occurring in erythrocyte membrane was selected. GpA is known to form a dimer[43] in membrane environments by interaction mainly between α-helical TM domain (73 ITLIIF-GVMAGVIGTILLISYGI 95) in which the GXXXG motif[44] (glycine zipper)[45] is considered to be important for dimerization. GpA is water soluble but tends to aggregate in water even in the presence of detergents. Bormann et al.[46] have reported that GpA migrated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as oligomers or dimers, which can be dissociated into monomers by peptides corresponding to the TM domain (Figure 4). The dissociation can thus be accounted for by direct binding of the peptides to the TM part of GpA. We utilized this method to evaluate the interaction with LSPs in place of the α-helical peptides on the basis of the SDS-PAGE analysis[47] and found that LSPs such as brevetoxin-B and yessotoxin as well as the tetracyclic ALP possessing benzyl ethers (Figure 3A)[28] induced dissociation of oligomeric GpA into its dimers and monomers. Under the same conditions, the ALPB series possessing benzyl ether moieties (**1b**, **2b**, and **3b**) were incubated (24 nmol, 1.5 mM) with GpA (2.6 pmol, 0.16 μM) in buffer containing 0.03% SDS (Figure 5A). As shown in lane 1 of Figure 5A, intact GpA exists as a mixture of oligomers and dimers. When GpA was treated with ALP1B (lane 2), oligomer bands decreased and dimer bands appeared. When GpA was treated with ALP4B (lane 3), the oligomer bands disappeared completely, and a considerable amount of monomers (38 kDa) as well as dimers emerged. Contrary to our expectation, ALP7B did not induce dissociation of GpA dimers on the SDS-PAGE (lane 4). For the ALP10B, even the dissociation of GpA oligomers did not occur, while a smaller amount of ALP10B (12 nmol, 0.75 mM) than ALP1B−7B (24 nmol, 1.5 mM) was incubated because of its low solubility in 0.03% SDS buffer (critical micelle concentration of SDS: ∼0.25%). Therefore, GpA was incubated with ALPB series in 2% SDS buffer (Figure 5B). As shown in lane 1 of Figure 5B, intact GpA exists predominantly as dimers with small amount of monomers. When GpA was treated with ALP1B (lane 2), ALP4B (lane 3), and ALP7B (lane 4), the monomer band increased with increasing the number of rings, whereas ALP10B (lane 5) exhibited no significant effect.

To investigate whether the ALPs interact with the α-helical TM domain of GpA, a peptide composed of 29 residues (GpA-TM: 70 EPEITLIIFGVMAGVIGTILLISYGIRRL 98) was

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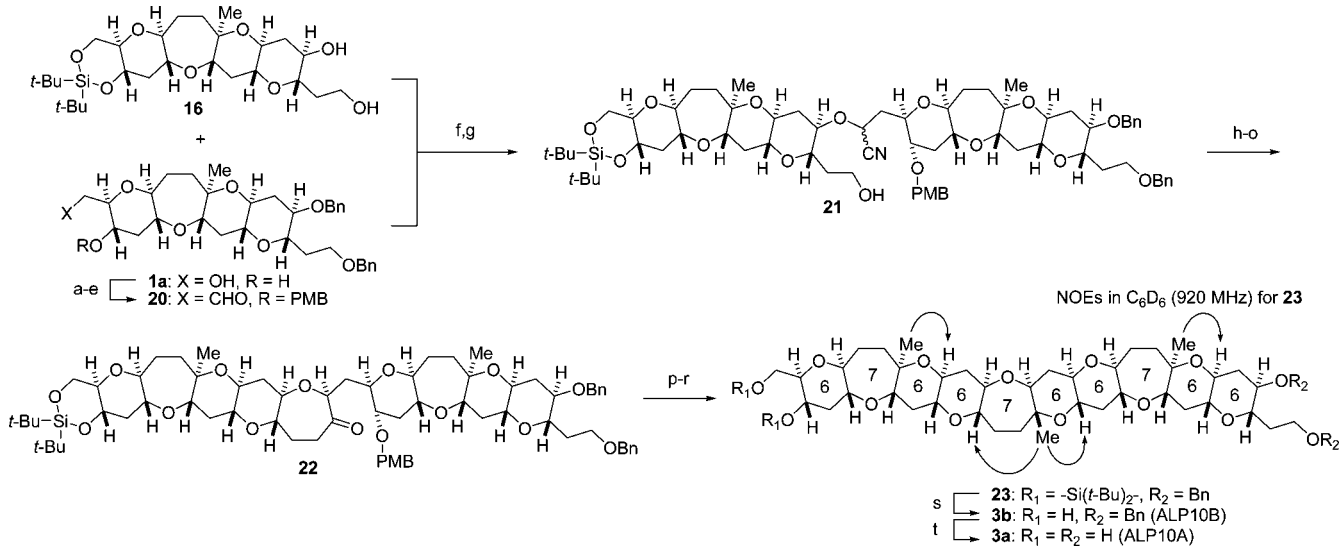
Scheme 4. Synthesis of the Decacyclic ALPs (**3**)[a]



[a] Reagents and conditions: (a) *p*-MeO-$C_6H_4CH(OMe)_2$, CSA, CH_2Cl_2, rt, 5 h, 92%; (b) DIBALH, CH_2Cl_2, −62 to −13 °C, 2 h; (c) MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min; (d) NaCN, 18-crown-6, DMF, 80 °C, 5 h, 94% (three steps); (e) DIBALH, CH_2Cl_2, −76 to −45 °C, 50 min, 93%; (f) $Sc(OTf)_3$, benzene, rt, 6.5 h, 85%; (g) TMSCN, $Sc(OTf)_3$, CH_2Cl_2, rt, 80 min, 61%; (h) *o*-NO_2−C_6H_4SeCN, *n*-Bu_3P, MS4A, THF, rt, 30 min; (i) *m*CPBA, 1,2-dichloroethane, rt, 15 min, then $NaHCO_3$, 60 °C, 24 min, 85% (two steps); (j) DIBALH, CH_2Cl_2, −83 to −50 °C, 35 min, 70%; (k) tetravinyltin, MeLi, THF, −78 to −63 °C, 30 min, 94%; (l) Dess−Martin periodinane, CH_2Cl_2, rt, 1.5 h, 99%; (m) Grubbs cat. (2nd generation) **11**, toluene, reflux, 15 min, 86%; (n) H_2, PtO_2, AcOEt, rt, 3.5 h, then Dess−Martin periodinane, CH_2Cl_2, rt, 30 min, 90%; (o) DBU, toluene, reflux, 21 h, 60%; (p) DDQ, H_2O, CH_2Cl_2, rt, 2 h, 83%; (q) EtSH, $Zn(OTf)_2$, CH_2Cl_2, 0 °C to rt, 1.5 h, 65%; (r) *m*CPBA, CH_2Cl_2, 0 °C, 20 min, then Me_3Al, −52 to −20 °C, 1 h, 96%; (s) HF·pyridine, THF, 0 °C to rt, 3.5 h, 98%; (t) H_2, $Pd(OH)_2$/C, THF, MeOH, rt, 4 days, 62%.

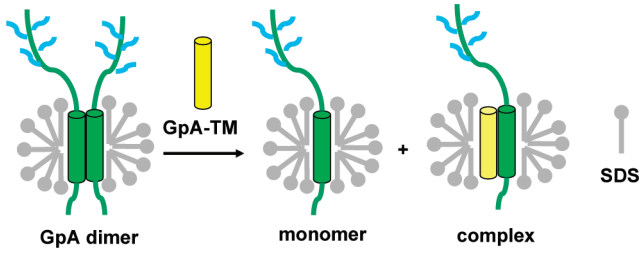


Figure 4. Illustrative pathways of dissociation of GpA dimer into monomer and GpA/GpA-TM complex.

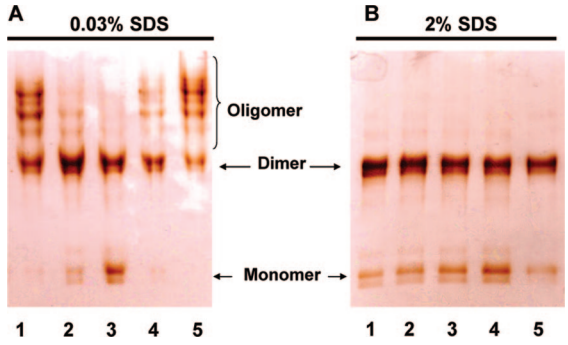


Figure 5. SDS-PAGE of GpA in the presence of ALPB series in SDS buffer. (A) GpA (2.6 pmol, 0.16 μM) alone (lane 1), in the presence of 24 nmol (1.5 mM) of ALP1B (lane 2), ALP4B (lane 3), ALP7B (lane 4), and 12 nmol of ALP10B (lane 5) in 0.03% SDS buffer. (B) GpA (2.6 pmol, 0.16 μM) alone (lane 1), in the presence of 24 nmol (1.5 mM) of ALP1B (lane 2), ALP4B (lane 3), ALP7B (lane 4), and ALP10B (lane 5) in 2% SDS buffer. GpA was visualized by silver staining.

synthesized using solid-phase methods,[48] and the interaction between ALP and GpA-TM was analyzed by using surface plasmon resonance (SPR) techniques.[49] The GpA-TM was coupled to the biosensor surface, and sensorgrams of ALPB series were recorded except for ALP10B because of the low

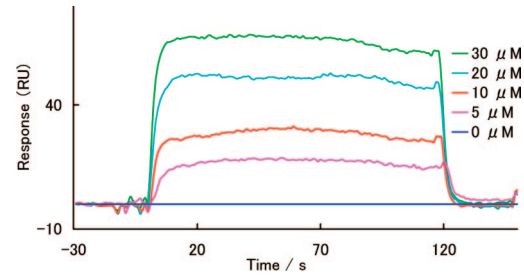


Figure 6. SPR sensorgram for the binding of different concentrations of ALP7B to GpA-TM.

Table 1. Dissociation Constants of ALPB Series against GpA-TM Determined by SPR

entry	ALP	K_D (μM)
1	ALP1B	2.4×10^3
2	ALP4B	2.4×10^2
3	ALP7B	48
4	ALP10B	ND[a]

[a] Not determined.

solubility in the running buffer containing DMSO (1%) and Tween20 (400 μM). Dose dependent changes in sensorgrams were observed with ALP7B (Figure 6) as well as ALP1B and ALP4B (Figures S1 and S2, Supporting Information). By comparing K_D values, affinities of ALPs to GpA-TM increased with increasing the number of rings (Table 1), which corresponded to the results of SDS-PAGE in Figure 5B.

Then GpA was treated with ALPA series with tetraols in 2% SDS buffer, however, no dissociation of the dimers was

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(49) (a) Fägerstam, L. G.; Frostell-Karlsson, Å.; Karlsson, R.; Persson, B.; Rönnberg, I. *J. Chromatogr.* **1992**, *597*, 397–410. (b) Johnsson, B.; Loefaas, S.; Lindquist, G. *Anal. Biochem.* **1991**, *198*, 268–277.

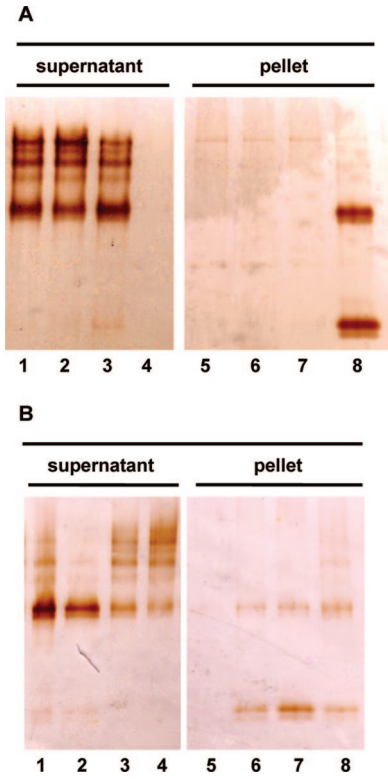

Figure 7. SDS-PAGE of GpA in the presence of ALPs in 0.03% SDS buffer. Supernatant of the centrifuged sample was loaded in lanes 1 to 4, and the residue dissolved in 6% SDS buffer was loaded in lanes 5 to 8, respectively. (A) GpA (2.6 pmol, 0.16 μM) in the presence of 24 nmol (1.5 mM) of ALP1A (lanes 1 and 5), ALP4A (lanes 2 and 6), ALP7A (lanes 3 and 7), and 12 nmol (0.75 mM) of ALP10A (lanes 4 and 8). (B) GpA (2.6 pmol, 0.16 μM) in the presence of 24 nmol (1.5 mM) of ALP1B (lanes 1 and 5), ALP4B (lanes 2 and 6), ALP7B (lanes 3 and 7), and 12 nmol (0.75 mM) of ALP10B (lanes 4 and 8). GpA was visualized by silver staining.

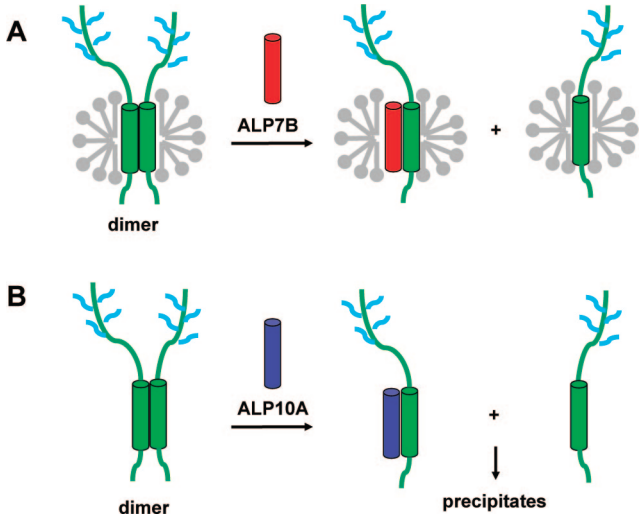

Figure 8. Hypothetical pathways of (A) dissociation of GpA dimers to monomers (ALP7B, 2% SDS) and (B) sedimentation of GpA (ALP10A, 0.03% SDS).

observed (Figure S3, Supporting Information). We postulated that ALPA series, both sides of which were functionalized with polar hydroxy groups, were prevented from permeation into SDS micelles. Therefore, GpA was incubated with ALPA series under the low concentration of SDS (0.03%), but no dissociation of GpA occurred (Figure S4, Supporting Information). Unexpectedly, an intriguing phenomenon was observed that some precipitates formed when GpA was treated with ALP10A. After centrifugation, the resulting precipitates were dissolved in 6% SDS buffer. The supernatant and the solubilized precipitates were subjected to SDS-PAGE, respectively (Figure 7A, lanes 4 and 8). The same procedure was also performed with ALP1A (lanes 1 and 5), ALP4A (lanes 2 and 6), and ALP7A (lanes 3 and 7). As shown in Figure 7A, only ALP10A turned out to induce sedimentation of GpA. The effect is dose dependent, and more than 150 times of ALP10A is required to elicit the activity (Figure S5, Supporting Information). It is noteworthy that the GpA pellet obtained by the treatment with ALP10A in 0.03% SDS buffer, was no longer soluble in a buffered aqueous solution in the absence of SDS, while intact GpA is highly soluble in water. Then, we ascertained whether the ALPB series cause precipitation of GpA under the low concentration of SDS (0.03%), and the same procedure as for ALPA series was performed with ALP1B (Figure 7B, lanes 1 and 5), ALP4B (lanes 2 and 6), ALP7B (lanes 3 and 7), and ALP10B (lanes 4 and 8). Among the ALPB series, ALP7B elicited the most potent precipitation-inducing activity, and the results are consistent with the GpA-dissociation activity in 2% SDS buffer (Figure 5B), while the activity of ALP7B is lower than that of ALP10A.

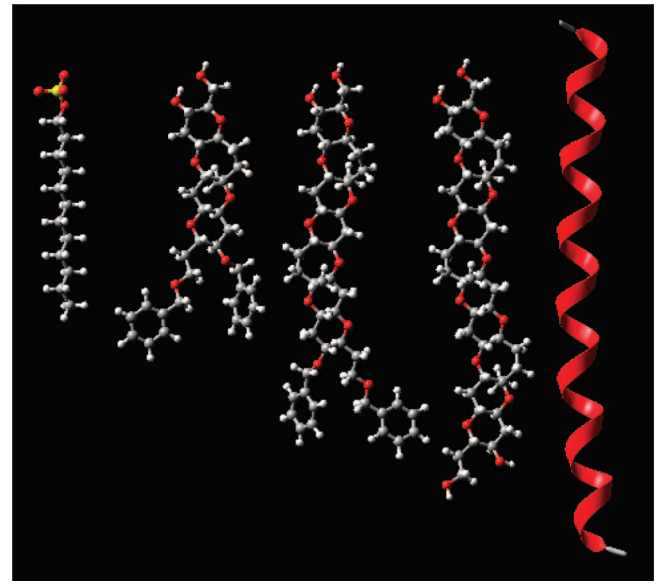

Figure 9. 3D structures of SDS, ALP4B, ALP7B, ALP10A, and GpA-TM (from left).

To examine whether the precipitate-inducing activity of ALP10A is specific for GpA, integrin $\alpha_1\beta_1$ was subjected to the same experiment. Integrin $\alpha_1\beta_1$ is known to form a heterodimer in membrane environment by interaction between α-helical TM domains (α_1: 966 LWVILLSAFAGLLLLMLLILALW 988; β_1: 966 IIPIVAGVVAGIVLIGLALLLIW 988) containing a glycine zipper motif (one of the G's is substituted with A or S).[50] Analogously, ALP10A induced sedimentation of integrin $\alpha_1\beta_1$ (Figure S6, Supporting Information). For bovine serum albumin, a water soluble protein without a TM domain, no precipitation formed by ALP10A in 0.03% SDS.

Discussion

The present SAR studies of the ALPs revealed that ALP7B elicited notable activity to induce dissociation of GpA dimers

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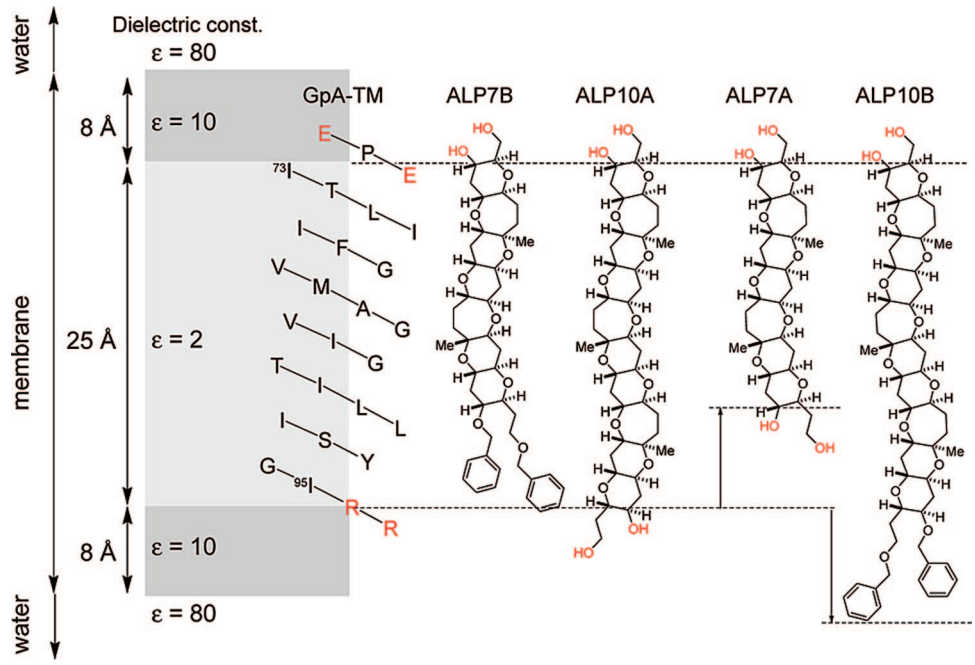

Figure 10. Comparison of the molecular length of ALPs, GpA-TM, and continuum electrostatic model of a biological membrane environment.[42]

to monomers in the presence of 2% SDS (in SDS micelles), while ALP10A exhibited intriguing phenomenon to induce sedimentation of GpA and integrin $\alpha_1\beta_1$ under the low concentration (0.03%) of SDS (Figure 8). ALP7B also induced precipitation of GpA in 0.03% SDS buffer but the efficacy is lower than ALP10A. Although the precise mechanism is unknown on the dissociation of GpA dimers or the formation of the precipitates containing GpA or integrins, both the dissociation- and precipitation-inducing activity against the TM proteins would be related to the interaction between ALPs and TM region of the proteins as supported by SPR experiments. We postulated that differences in efficacy among the ALPs can be correlated with the concept of "hydrophobic matching",[51] since SDS micelles are expected to provide membrane environments similar to those of lipid bilayers, particularly for hydrophobic interactions with TM regions. Comparison of the molecular lengths of the ALPs and TM region of GpA (GpA-TM) was performed by a MacroModel program (Figure 9) as schematically illustrated in Figure 10. The molecular length was also compared with a five-slab of continuum electrostatic model of a biological membrane environment reported by Sengupta et al.,[52] in which the membrane model is composed of a low dielectric constant ($\epsilon = 2$) region corresponding to the alkyl chains of lipids, high dielectric constant ($\epsilon = 10$) region corresponding to the polar head groups of lipids, and bulk water ($\epsilon = 80$) phase. As shown in Figure 10, it is obvious that the length of the hydrophobic region of ALP7B including the benzyl ethers (ca. 25 Å) matches that of low dielectric constant region of membrane when the polar hydroxy groups are placed into the slab of high dielectric constant. The hydrophobic nature of the benzyl groups is also considered to assist insertion of ALP7B into lipid membranes. In analogy with other membrane proteins, the amino acid sequence of GpA-TM matches the continuum electrostatic model; thus, the hydrophobic region (I73−I95) lies between the polar amino acid residues (E70, E72 and R96, R97) residing at both ends of the α-helix. For ALP10A, not only the hydrophobic (polycyclic ether skeleton) but also hydrophilic (hydroxy groups) region can be placed in the five slab areas in a similar manner to that of GpA-TM. On the other hand, the length of the hydrophobic region of ALP7A is not long enough, and that of ALP10B is too long in comparison with that of GpA-TM, which could account for their weak dissociation- or precipitation-inducing activity. Looking back at the structures of naturally occurring LSPs (Figure 1), we noticed that ciguatoxins match the model, and relatively smaller but biologically active LSPs, such as brevenal (pentacyclic) and gambierol (octacyclic), possess somewhat longer side chains than other LSPs. It would be correlated to the concept of hydrophobic matching when the LSPs bind to the target proteins in the lipid bilayer membranes. Although the concept of hydrophobic matching is still controversial on the interaction of LSPs and TM proteins, it might be a clue to understanding the mode of action of naturally occurring LSPs and also a guiding principle to design ALPs possessing potent affinities with TM proteins. Besides these studies for elucidating the molecular mechanism underlying the powerful toxicity of natural LSPs, the characteristic property that some ALPs such as ALP7B and ALP10A interact, dissociate, or precipitate TM proteins can be utilized to fish out membrane-integral proteins from cell lysates in proteomics and related areas.

Although there are few detailed proposals on the molecular recognition between the TM proteins and the LSPs to date, it has been suggested that (i) van der Waals interaction including CH/π interaction[53] might be an important factor because of the hydrophobic nature of the TM domain, and (ii) hydrogen bonding between skeletal oxygen atoms of LSPs and the OH/NH groups of the α-helical peptides stabilizes their complexation

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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).[26] On the other hand, a glycine zipper is expected to be one of the candidates for the interacting motifs because of the common structural feature of GpA and integrins.[28] The glycine zipper motif is abundantly found in homooligomeric domains of channel complexes: potassium channel pore-lining helices (KcsA),[54] mechanosensitive channel of large conductance (MscL),[55] vacuolating toxin anion selective channel (VacA),[56] mechanosensitive channel of small conductance (MscS),[57] and also in homooligomeric domains of G-protein-coupled receptors (GPCRs) such as α-factor receptors,[58] ErbB,[59] and CCK4.[60] In the glycine zipper motif, weak hydrogen bonds between the $C_\alpha-H$ of glycine and a carbonyl oxygen ($C_\alpha-H\cdots O$)[61] were suggested to play an important role in stabilizing the dimeric structure; therefore, the $C_\alpha-H$ of glycine could be also expected to be a hydrogen bond donor toward skeletal oxygen atoms of LSPs. In addition to the weak hydrogen bonding, dipole−dipole interactions between weakly polarized functional groups presented in the hydrophobic environment would be an important factor of the molecular recognition between LSPs and TM proteins.

Conclusion

In conclusion, aritificial ladder-shaped polyethers (ALPs) composed of an iterative 6/7/6 ring system with different molecular lengths (tetracyclic, heptacyclic, and decacyclic ethers) were systematically synthesized on the basis of the convergent strategy via α-cyano ethers. The interaction with transmembrane (TM) proteins, glycophorin A (GpA) and integrin $\alpha_1\beta_1$, were evaluated by SDS-PAGE and partly by SPR. The heptacyclic ether (ALP7B) exhibited notable activity to induce dissociation of GpA dimers to monomers in the presence of 2% SDS. The K_D value between ALP7B and a peptide composed of 29 residues corresponding to TM domain of GpA (GpA-TM) was determined to be 48 μM by SPR. The decacyclic ether (ALP10A) exhibited an intriguing phenomenon to induce precipitation of GpA dose-dependently under the low concentration of SDS (0.03%). ALP10A also induced precipitation of integrin $\alpha_1\beta_1$ which is also known to form heterodimers in the lipid bilayer membranes. These results can be related to the concept of hydrophobic matching; that is, the length of the hydrophobic region including the side chains of ALP7B and ALP10A is ca. 25 Å, which matches the lengths of the hydrophobic region of α-helical TM proteins. Preparation of the focused library of ALPs possessing different ring size and functional groups and evaluation of their biological activities and the interaction with various TM proteins are in progress in our laboratory.

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Supporting Information Available: Experimental section including the synthesis of the ALPs and copies of the NMR spectra of new compounds, SDS-PAGE, and SPR analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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