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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 3181–3186

Design and synthesis of simplified polycyclic ethers and evaluation of their interaction with an α-helical peptide as a model of target proteins

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Received 10 February 2007; revised 3 March 2007; accepted 8 March 2007
Available online 12 March 2007

Abstract—Two simplified pentacyclic ethers, having 6/7/6/6/7 and 6/7/6/7/7 ring systems, were synthesized. A convergent route based on the Suzuki–Miyaura cross-coupling strategy was applied to synthesize these two compounds. Interactions between α-helical peptide, melittin, and synthesized pentacyclic ethers were evaluated by circular dichroism (CD) spectroscopy. Interestingly, only the polycyclic ether having a 6/7/6/7/7 ring stabilized the α-helical structure of melittin. This result indicated that a ring fusion manner of the polycyclic structure is important to recognize membrane proteins.
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Since the structure of brevetoxin B, a redtide toxin of Florida red tides, was first reported by Nakanishi and co-workers in 1981,[1] a large number of fused polycyclic ether marine toxins have been isolated and characterized.[2] The intriguing structures of these marine toxins, along with their potent and diverse biological activities, have stimulated the interest of both chemists and biologists. Because of the scarcity of polycyclic ethers from natural sources, the target receptor protein has been identified only for brevetoxins[3] and ciguatoxins.[4] These toxins share a common binding site of voltage-sensitive sodium channels (VSSCs), designated as site 5.[5] The toxicity of these molecules is thought to be associated with its conformational flexibility[2b] but detailed mechanism of these molecular recognitions remains unclear.

Previously, our group reported that brevetoxins A and B inhibit the Ca^{2+} influx of C6 glioma cells induced by the polycyclic ether toxin, maitotoxin.[6,7] Although this inhibition needed high concentration of brevetoxins, this result suggests that brevetoxins weakly bind to maitotoxin-target molecules, which are thought to be membrane proteins different from VSSCs.[8] Considering this result, we hypothesize a general recognition mechanism between polycyclic ethers and membrane proteins.[9] Recently, Murata, Oishi, and co-workers reported that a natural polycyclic ether, yessotoxin, interacts with a transmembrane protein, glycophorin A.[10] They also reported the interaction between a synthesized tetracyclic ether and the same protein but there was no study about a ring fusion system.[9c]

The importance of continuous oxepane rings of brevetoxin B was reported[11] and this ring fusion system is thought to give flexibility to the molecule. Recently, Martín and co-workers reported that two fused oxepane rings provide milli-second scale conformational change.[12] Based on these facts, we focused on the continuous oxepane rings to evaluate the skeletal factor of polycyclic ethers and designed two simplified pentacyclic ether compounds, **1** (6/7/6/6/7) and **2** (6/7/6/7/7) (Fig. 1). Polycyclic ether **2** has flexible two fused oxepane rings. An alkyl chain and a benzyl ether in a left hemisphere were designed to give hydrophobicity considering the amphiphilic nature of natural polycyclic ethers. In this Letter, we report convergent syntheses of **1** and **2**, and their interaction with the α-helix part of melittin.

Numerous efforts have been directed toward the synthesis of polycylclic ethers,[13] among which Sasaki and others of our group developed the Suzuki–Miyaura

Keywords: Polycyclic ether; Simplified ring system; Suzuki–Miyaura cross coupling; Convergent synthesis; α-Helix.
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doi:10.1016/j.tetlet.2007.03.041

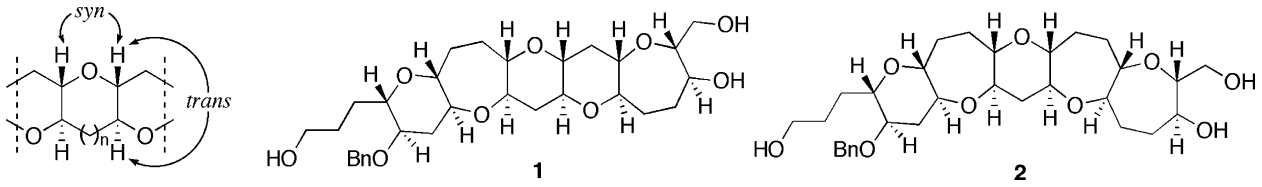


Figure 1. Common structural feature of polycyclic ether toxins and the structure of simplified polycyclic ethers **1** (6/7/6/6/7) and **2** (6/7/6/7/7).

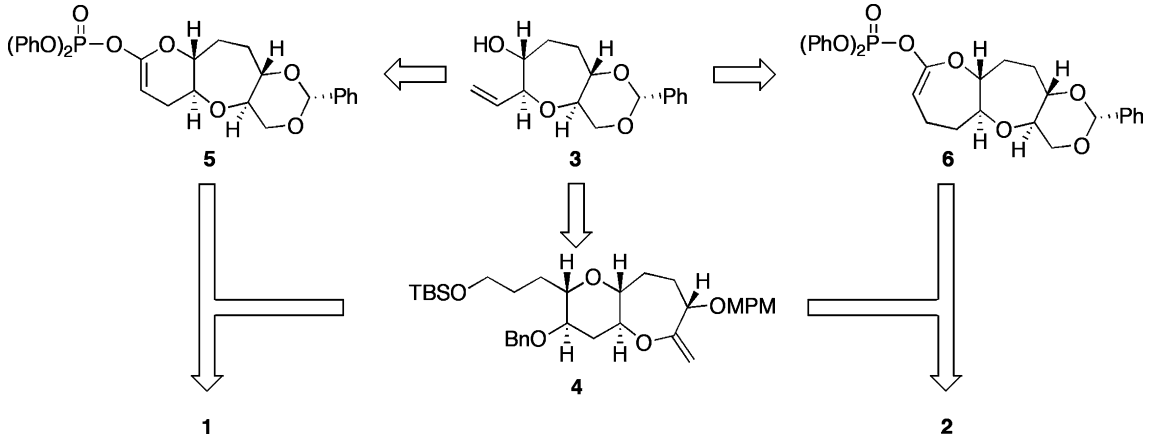
cross-coupling strategy.[14–16] Two pentacyclic ethers were synthesized by combination of three segments, **4**–**6**, using this strategy (Scheme 1). To expedite the synthesis, these segments were prepared from the same oxepane **3**, which could derive the following known scheme.[17]

The synthesis of **4** and **5** is outlined in Scheme 2. The side chain of oxepane **3** was homologated to unsaturated ester **7** by ozonolysis and Wittig reaction. Hydrogenation of **7** followed by lactonization gave lactone **8**. These steps followed the known procedure.[18] Treatment of **8** with KHMDS, HMPA, and $(PhO)_2P(O)Cl$ gave ketene acetal phosphate **5** (73% yield), as one of the right segments.

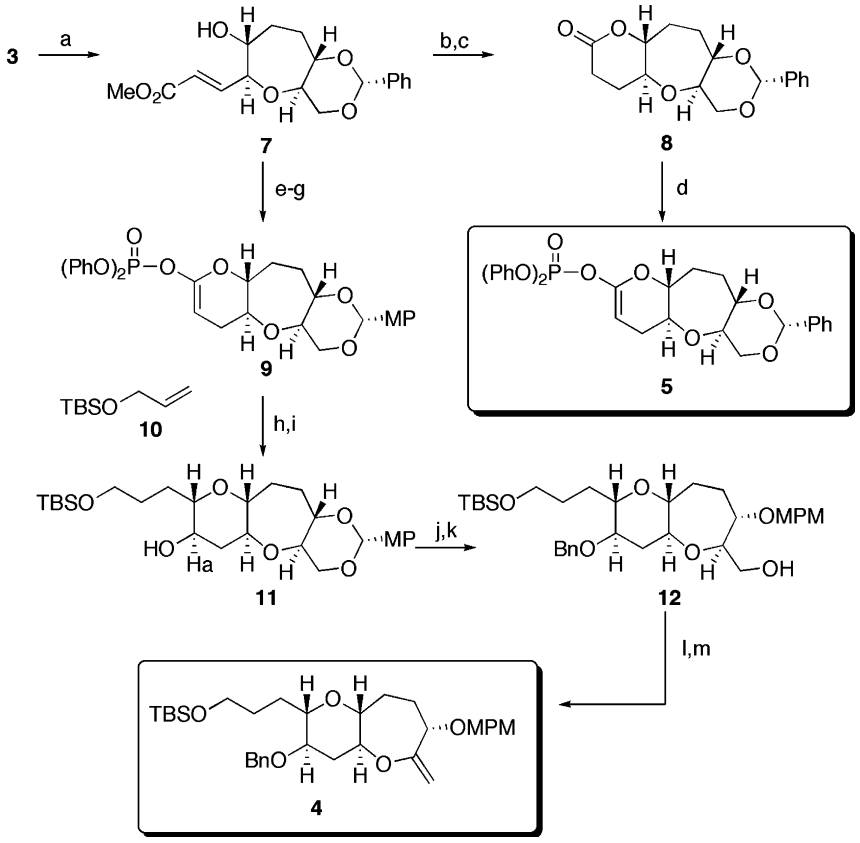
Preparation of the left segment **4** was started from the same hydroxyester **7**. Simultaneous hydrogenation and removal of benzylidene were achieved by treatment of **7** with 10% Pd/C in THF under hydrogen. Following reprotection and lactonization gave a lactone (86% yield) and the treatment of the lactone with KHMDS, HMPA, and $(PhO)_2P(O)Cl$ gave ketene acetal phosphate **9** in 70% yield. Suzuki–Miyaura cross-coupling of **9** with alkylborane derived from olefin **10**,[19] followed by stereoselective hydroboration–oxidation, provided alcohol **11** in 77% for the two steps. The stereochemistry of **11** was confirmed by proton coupling constant analysis of the corresponding acetate derivative ($^3J_{9,10} = 9.6$ Hz, $^3J_{8eq,9} = 4.8$ Hz, $^3J_{8ax,9} = 11.2$ Hz). Protection of **11** as the benzyl ether followed by regioselective cleavage of *p*-methoxybenzylidene acetal with DIBAL–H gave alcohol **12** in 76% yield for the two steps. Iodination of **12** under standard conditions followed by treatment with KO*t*-Bu in THF furnished the left segment **4** in 93% yield for the two steps.

Synthesis of another right segment **6** also began with the same oxepane **3** (Scheme 3). Protection of a hydroxy group as the MPM ether followed by hydroboration–oxidation led to alcohol **13** in 96% for the two steps. Swern oxidation[20] of **13** followed by Wittig reaction led to unsaturated ester **14** in 94% for the two steps. Removal of the MPM ether by DDQ gave rise to hydroxy ester **15** in 94% yield and hydrogenation and saponification furnished hydroxy acid **16** in 91% yield for the two steps. Lactonization following the Yamaguchi protocol[21] gave lactone **17** 88% yield.[22] Finally, the treatment of lactone **17** with KHMDS, HMPA, and $(PhO)_2P(O)Cl$ furnished ketene acetal phosphate **6** (96% yield) as another right segment.

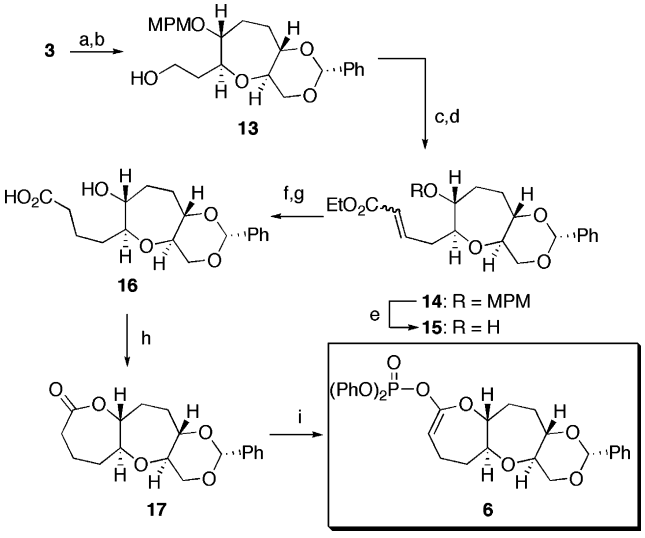
The synthesis of **1** and **2** is outlined in Scheme 4. Stereoselective hydroboration of exocyclic enol ether **4** with 9-BBN produced alkylborane,[23] which was in situ reacted with ketene acetal phosphate **5** in the presence of aqueous Cs_2CO_3 and a catalytic amount of $PdCl_2(dppf)\cdot CH_2Cl_2$, giving rise to cross-coupling product **18** in 89% yield from **4**. Stereoselective hydroboration–oxidation of **18** furnished alcohol **19** in 88% yield. Oxidation of the resultant hydroxyl group with TPAP/NMO[24] led to ketone **20** in 98% yield. The relative configuration of **20** was unambiguously confirmed by NOE analysis as shown. Removal of the MPM group followed by treatment with EtSH and $Zn(OTf)_2$ furnished mixed thioacetal **21** in 75% for the two steps.[25] Finally, radical reduction with Ph_3SnH in the presence of AIBN gave rise to pentacyclic ether **1** in high yield.[26]



Scheme 1. Synthetic plan of simplified polycyclic ethers.



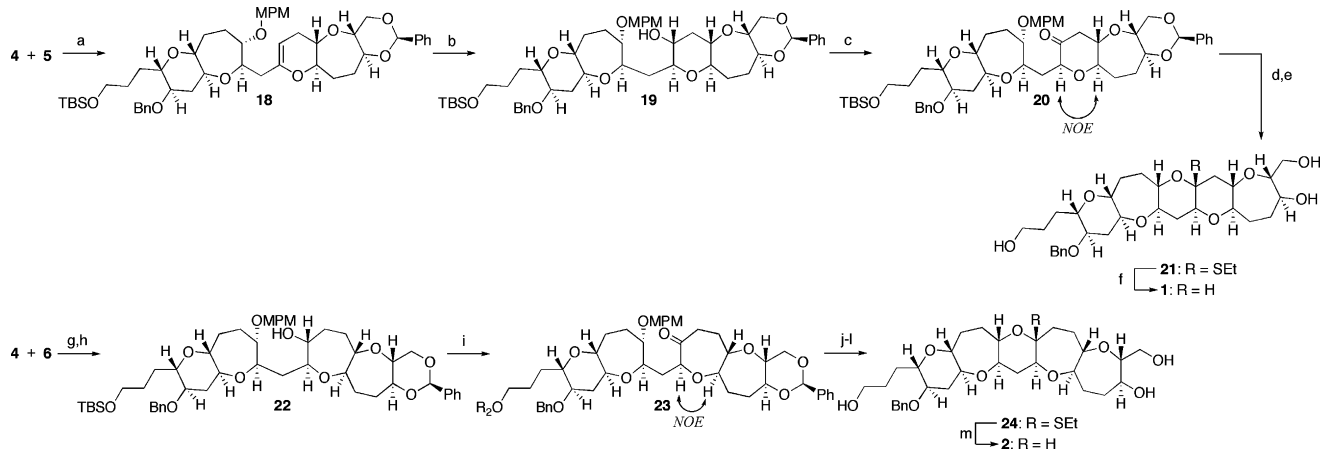
Scheme 2. Reagents and conditions: (a) O_3, CH_2Cl_2, −78 °C; then SMe_2; then Ph_3PCHCO_2Me, rt, 98%; (b) H_2, 10% Pd/C, EtOAc, rt, 93%; (c) PPTS, benzene, reflux, 84%; (d) KHMDS, $(PhO)_2P(O)Cl$, HMPA, THF, −78 °C, 73%; (e) H_2, 10% Pd/C, THF, rt; (f) *p*-$MeOC_6H_4CH(OMe)_2$, CSA, benzene, reflux, 86% (two steps); (g) KHMDS, $(PhO)_2P(O)Cl$, HMPA, THF, −78 °C, 70%; (h) **10**, 9-BBN, THF, 0 °C; then aq Cs_2CO_3, **9**, $PdCl_2(dppf)\cdot CH_2Cl_2$, DMF, 45 °C, 87%; (i) thexylborane, THF, 0 °C; then aq NaOH, 30% H_2O_2, 0 °C, 88%, (j) KO*t*-Bu, THF, 0 °C; then BnBr, 89%; (k) DIBAL–H, CH_2Cl_2, −40 °C, 85%; (l) I_2, PPh_3, imidazole, benzene, rt, 95%; (m) KO*t*-Bu, THF, 0 °C, 98%.



Scheme 3. Reagents and conditions: (a) KO*t*-Bu, THF, 0 °C; then MPMCl, 99%; (b) 9-BBN, THF, 0 °C; then aq NaOH, 30% H_2O_2, 0 °C, 97%; (c) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C; then Et_3N, −78 °C to rt; (d) Ph_3PCHCO_2Et, THF, rt, 94% (two steps); (e) DDQ, CH_2Cl_2-buffer (pH 7.0), rt, 94%; (f) H_2, 10% Pd/C, EtOAc, rt; (g) $LiOH\cdot H_2O$, THF–H_2O, 45 °C, 91% (two steps); (h) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, rt; then DMAP, toluene, 100 °C, 88%; (i) KHMDS, $(PhO)_2P(O)Cl$, HMPA, THF, −78 °C, 96%.

Another pentacyclic ether **2** was synthesized by an analogous process. Hydroboration of **4** with 9-BBN followed by coupling with **6** proceeded in 91% yield from **4** and hydroboration–oxidation of the coupling product proceeded stereoselectively to yield alcohol **22** in 88% yield. Oxidation of the resultant hydroxyl led to ketone **23** in 81% yield. The relative configuration of **23** was unambiguously confirmed by NOE analysis in the same manner as shown. Three-step conversion provided mixed thioacetal **24** in 81% overall yield. Finally, radical reduction under the above conditions gave rise to another pentacyclic ether **2**[27] in 94% yield. The relative configuration of **1** and **2** was unambiguously confirmed by NOE analysis of acetate derivatives **25** and **26** (Fig. 2).

Because the only known target molecule of polycyclic ethers, VSSCs, is comprised 24 transmembrane helices, we assumed that the interacting motifs of them are membrane-integrated α-helices. Therefore, interaction of two simplified polycyclic ethers, **1** and **2**, with an α-helical peptide, melittin, was evaluated by CD spectroscopy.[28] Melittin is the principal venom component of the European honey bee, *Apis meliffella*.[29] This peptide is composed of 26 amino acid residues and is known that the secondary structure of melittin mainly consists of rather unstable α-helix in methanol.[30] So we used this solvent as the medium to investigate change in the CD



Scheme 4. Reagents and conditions: (a) **4**, 9-BBN, THF, 0 °C; then aq Cs_2CO_3, **5**, $PdCl_2(dppf)\cdot CH_2Cl_2$, DMF, 45 °C, 89%; (b) $BH_3\cdot$THF, THF, 0 °C; then aq NaOH, 30% H_2O_2, 0 °C, 88%; (c) TPAP, NMO, 4 Å molecular sieves, rt, 98%; (d) DDQ, CH_2Cl_2-buffer (pH 7.0), rt; (e) EtSH, $Zn(OTf)_2$, CH_2Cl_2, rt, 75% (two steps); (f) Ph_3SnH, AIBN, toluene, reflux, quant; (g) **4**, 9-BBN, THF, 0 °C; then aq Cs_2CO_3, **6**, $PdCl_2(dppf)\cdot CH_2Cl_2$, DMF, 45 °C, 91%; (h) thexylborane, THF, 0 °C; then aq NaOH, 30% H_2O_2, 0 °C, 81%; (i) TPAP, NMO, 4 Å molecular sieves, rt, 94%; (j) TBAF, THF, rt, 88%; (k) DDQ, CH_2Cl_2-buffer (pH 7.0), rt; (l) EtSH, $Zn(OTf)_2$, CH_2Cl_2, rt, 93% (two steps); (m) Ph_3SnH, AIBN, toluene, reflux, 94%.

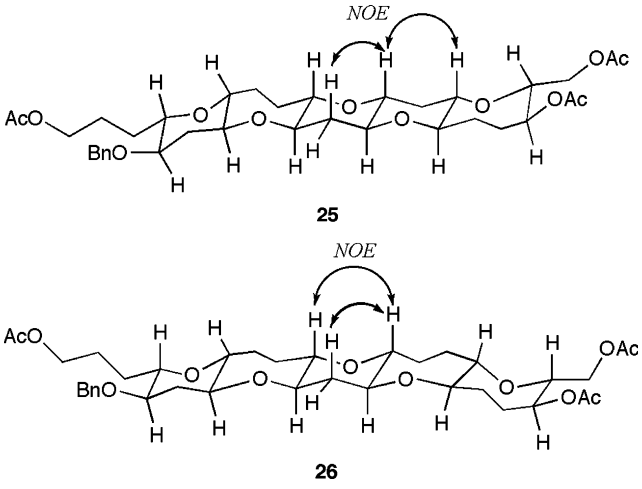


Figure 2. Stereochemical confirmation of pentacyclic ethers **25** and **26**.

spectra intensity of melittin coexisting **1** and **2**. As shown in Figure 3, the CD spectrum showed little conformational change of melittin with the existence of **1**, whereas, the existence of **2** provides a marked increase of α-helicity in melittin (minimum at 208 and 222 nm).[31] Figure 3b shows the relationship between the concentration of simplified polycyclic ethers and α-helicity of melittin. The α-helicity of melittin depended on the concentration of **2**. These results suggest that there is mutual interaction between the α-helix and the polycyclic ether structures. Thus, the flexibility of polycyclic ether is thought to be an important factor of these interactions.

In conclusion, two pentacyclic ethers **1** and **2** were designed and synthesized using the Suzuki–Miyaura cross-coupling strategy with the aim to evaluate the skeletal factor of their interaction with α-helical peptides. As a result, the stronger interaction between pentacyclic ether **2** and α-helical peptide, melittin, was observed.

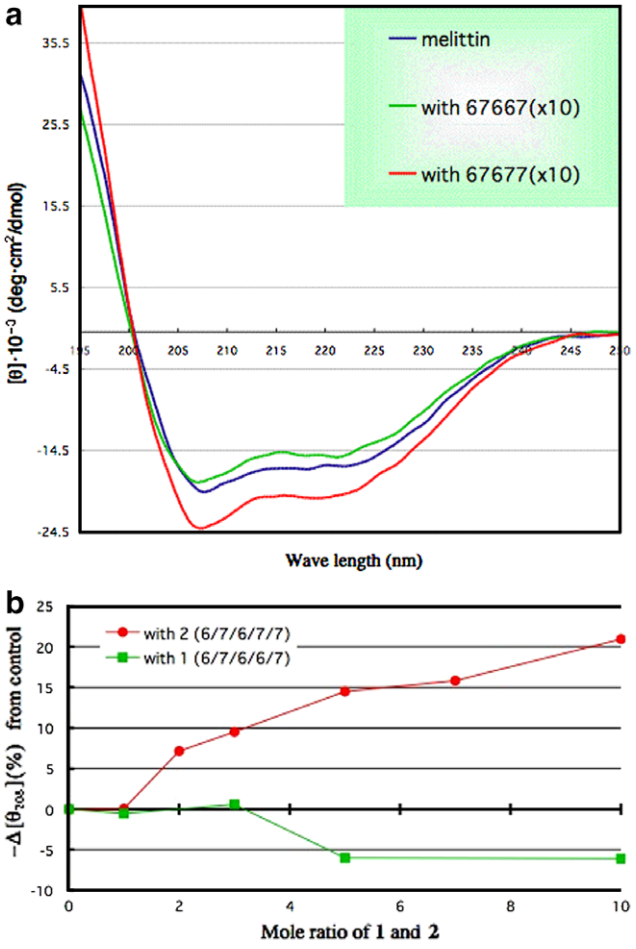


Figure 3. (a) CD spectra of melittin (10 μM) in the absence or the presence of **1** and **2** (100 μM) in methanol at 25 °C; (b) ellipticity change at 208 nm for melittin (10 μM) in the presence of various amounts of **1** and **2** (10–100 μM).

Structural difference between **1** and **2** is only the size of the ether ring. Thus, this result indicates the

importance of the ring arrangement of the polycyclic ether core to interact with proteins. In order to accumulate further knowledge about the mechanism of these interactions, the synthesis of elongated polycyclic ethers is in progress.

Acknowledgments

We are grateful to Professor M. Satake in our laboratory for a valuable discussion. Mr. S. Motoi contributed to this study in an earlier stage. This work was financially supported by CREST, Japan Science and Technology Agency (JST) and 21st century COE program. A fellowship (to M.S.) from the Ajinomoto Scholarship Foundation is also gratefully acknowledged.

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26. *Data for* **1**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.26 (m, 5H), 4.63 (d, $J = 11.4$ Hz, 1H), 4.42 (d, $J = 11.4$ Hz, 1H), 3.85–3.80 (m, 1H), 3.68 (dd, $J = 11.2$, 4.2 Hz, 1H), 3.65–3.53 (m, 3H), 3.49–3.44 (m, 1H), 3.32–3.24 (m, 2H),

3.23–3.12 (m, 5H), 3.09–3.04 (m, 1H), 3.01–2.91 (m, 2H), 2.55 (dt, $J = 11.8$, 3.9 Hz, 1H), 2.32–2.26 (m, 2H), 2.07–1.94 (m, 3H), 1.94–1.71 (m, 9H), 1.70–1.62 (m, 2H), 1.51–1.35 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.9, 128.5, 127.9, 127.8, 86.2, 82.4, 81.9, 81.6, 81.4, 80.8, 79.3, 79.1, 76.6 (×2), 76.2, 71.2, 70.9, 64.7, 63.0, 37.0 (×2), 36.9, 30.0, 29.2, 29.1 (×2), 28.6, 26.5; HRMS (FAB) calcd for $C_{30}H_{44}O_9Na$ [$(M+Na)^+$] 571.2883. Found: 571.2872.

27. *Data for* **2**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.26 (m, 5H), 4.62 (d, $J = 11.4$ Hz, 1H), 4.42 (d, $J = 11.4$ Hz, 1H), 3.72 (ddd, $J = 11.3$, 8.0, 4.2 Hz, 1H), 3.67–3.51 (m, 4H), 3.48–3.35 (m, 3H), 3.27–3.12 (m, 6H), 3.09 (dt, $J = 9.3$, 6.1 Hz, 1H), 3.00 (dt, $J = 8.9$, 3.9 Hz, 1H), 2.52 (dt, $J = 11.8$, 3.9 Hz, 1H), 2.31 (t, $J = 5.9$ Hz, 1H), 2.28 (dt, $J = 11.9$, 4.3 Hz, 1H), 2.09–1.74 (m, 14H), 1.71–1.62 (m, 3H), 1.52–1.34 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.0, 128.4, 127.8 (×2), 86.5, 84.9, 82.8, 81.7, 81.6, 81.2, 80.8, 80.4, 79.3, 79.0, 76.3, 71.9, 70.9, 64.7, 62.9, 38.8, 36.9, 30.2, 29.9, 29.2, 29.1, 28.7, 28.6 (×2), 28.4; HRMS (FAB) calcd for $C_{31}H_{46}O_9Na$ [$(M+Na)^+$] 585.3040. Found: 585.3058.
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31. The degree (fraction, f) of helicity was calculated from $f = -(\theta_{222} + 2340)/30{,}300$.[28a] The percentages of helicity of melittin were increased from 62% to 73% with the existence of **2**.