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Structure–activity relationship studies of gymnocin-A

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Abstract—The structural elements required for cytotoxicity of gymnocin-A, a polycyclic ether isolated from the red tide-forming dinoflagellate, *Karenia mikimotoi*, were investigated by the total synthesis and evaluation of the structural analogues. The results of the structure–activity relationship studies indicated that the α,β-unsaturated aldehyde functionality of the side chain as well as the molecular length were needed for exhibiting the cytotoxicity of gymnocin-A.

The polycyclic ether natural products, exemplified by brevetoxins and ciguatoxins, exhibit diverse biological activities with extreme potency in spite of the common structural motif.[1] Due to their unique structural features and biological properties, polycyclic ethers have attracted the attention of not only chemical but also biological communities. Therefore, new strategies and methodologies for constructing the polycyclic ether system have been explored and established to date.[2] Accumulation of these studies culminated in the accomplishment of the total synthesis of some natural products.[3] However, there are only a few reports concerning the structure–activity relationship studies of polycyclic ethers.[4,5]

Gymnocin-A (**1**, Fig. 1) is a polycyclic ether toxin, isolated from the notorious red tide-forming dinoflagellate, *Karenia mikimotoi*, which is a representative species that causes devastating damage worldwide.[6] Structurally, it is characterized by 14 contiguous and saturated ether rings and a 2-methyl-2-butenal side chain. The toxin molecule exhibits in vitro cytotoxicity against P388 murine leukemia cells with IC_{50} value of 1.3 μM. To date, the biological mechanism of action remains unknown. Several congeners of **1**, including gymnocin-B ($IC_{50} = 1.47$ μM) (Fig. 1),[7] have also been isolated and some of them displayed cytotoxicity far stronger than that of **1**. We have already accomplished the first total synthesis of gymnocin-A by using the Suzuki–Miyaura

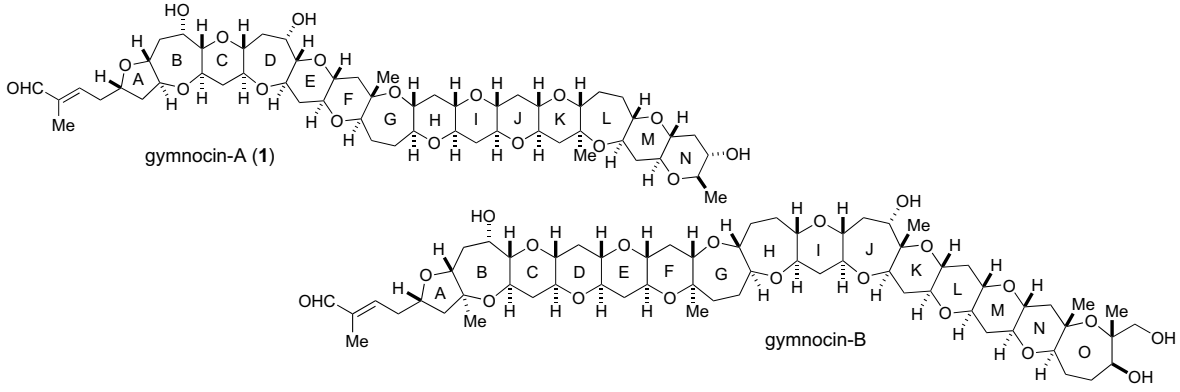

Figure 1. Structures of gymnocin-A (**1**) and gymnocin-B.

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coupling-based methodology.[8,9] After the completion of the total synthesis, we focused our attention on elucidation of the structural elements required for cytotoxicity of gymnocin-A. We describe herein the total synthesis of a series of structural analogues (**5**–**10**, Fig. 2) of gymnocin-A and evaluation of their cytotoxicity against the P388 murine leukemia cell line.

Our convergent total synthesis is well-suited for the preparation of a diverse set of gymnocin-A analogues with modified side chain and molecular length. We have reported that analogues **2**–**4** (Fig. 2) showed no detectable cytotoxicity against P388 even at a concentration of 100 μM.[8d,10] In order to investigate whether the 2-methyl-2-butenal side chain is sufficient for exerting cytotoxicity, analogues **5**–**8** were synthesized. Alcohol **11**, an advanced intermediate in our total synthesis,[8c,d] was converted to α,β-unsaturated ester **12** in two steps (Scheme 1). Subsequent deprotection of the TES groups with TASF[11] yielded triol **5** in a quantitative yield. Hydrolysis of **5** with $Ba(OH)_2$ provided carboxylic acid **6**. Analogues **7** and **8** were also prepared starting with alcohol **11**. Oxidation of **11** with TPAP[12] and Wittig reaction led to **13**. DIBALH reduction and global

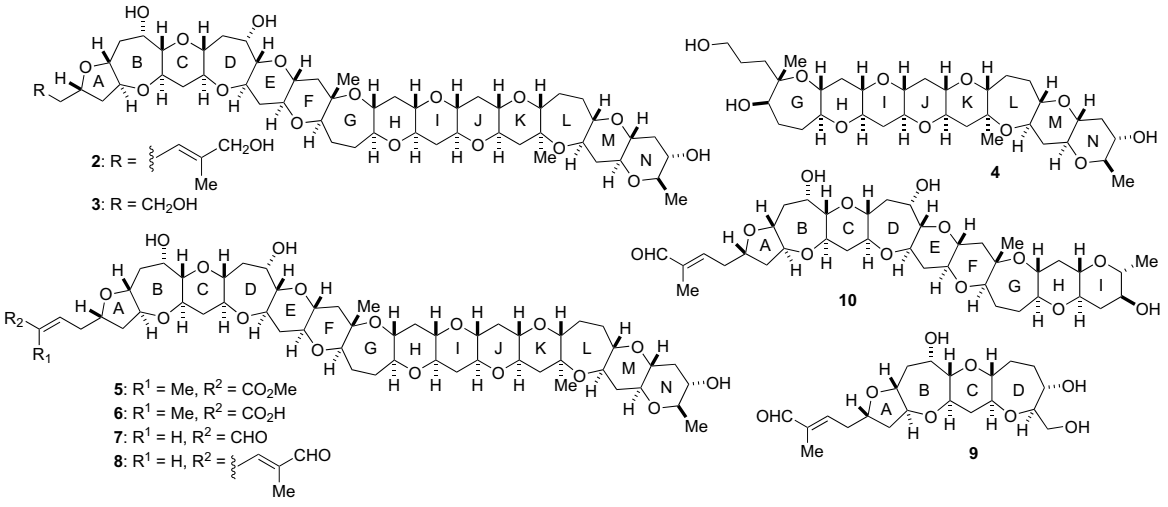
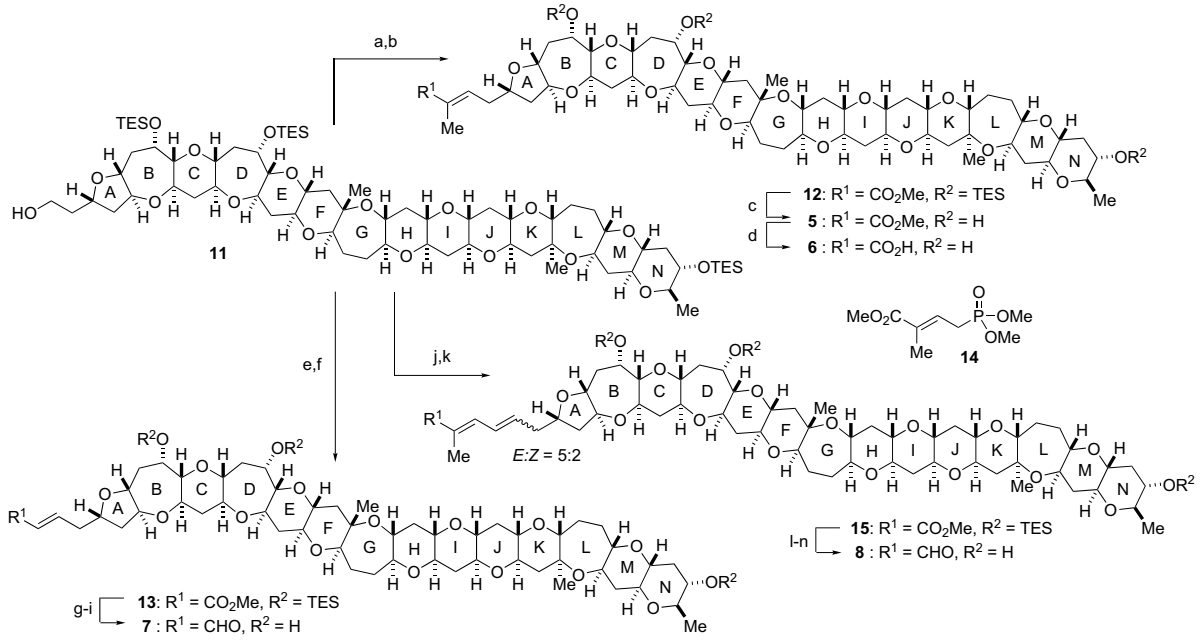
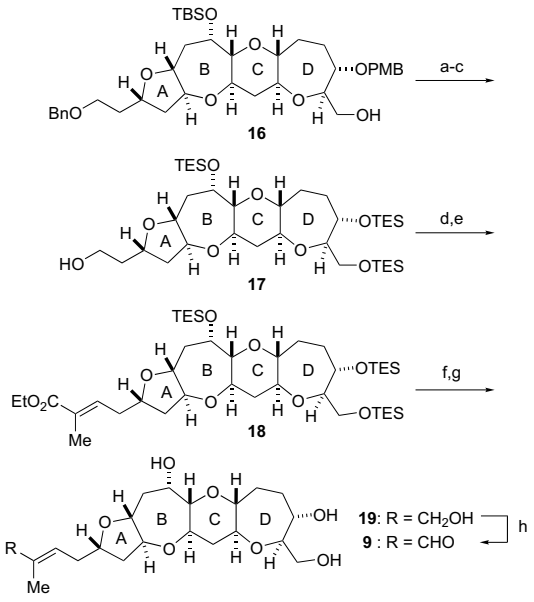


Figure 2. Structures of gymnocin-A analogues **2**–**10**.



Scheme 1. Reagents and conditions: (a) TPAP, NMO, 4 Å MS, CH_2Cl_2, rt; (b) $Ph_3P{=}C(Me)COOMe$, CH_2Cl_2, rt, 88% (two steps); (c) TASF, THF/DMF, rt, quant.; (d) $Ba(OH)_2{\cdot}8H_2O$, dioxane/H_2O, rt, quant.; (e) TPAP, NMO, 4 Å MS, CH_2Cl_2, rt; (f) $Ph_3P{=}CHCOOMe$, CH_2Cl_2, rt; (g) DIBALH, CH_2Cl_2, −78 °C, 66% (three steps); (h) TASF, THF/DMF, rt; (i) MnO_2, $CHCl_3$, rt, 91% (two steps); (j) TPAP, NMO, 4 Å MS, CH_2Cl_2, rt; (k) **14**, LiHMDS, THF, 0 °C → rt, 94% (two steps); (l) DIBALH, CH_2Cl_2, −78 °C; (m) TASF, THF/DMF, rt; (n) MnO_2, $CHCl_3$, rt, 72% (three steps).

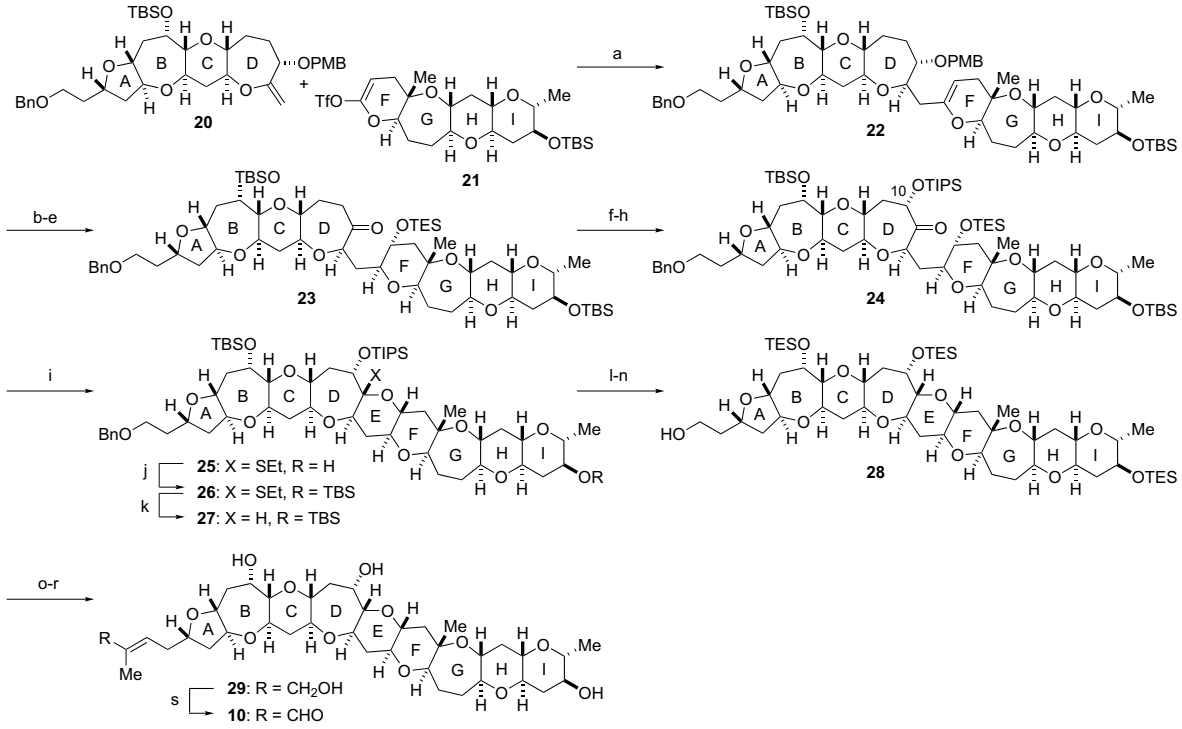


Scheme 2. Reagents and conditions: (a) *p*-TsOH, MeOH, 64 °C; (b) TESOTf, 2,6-lutidine, CH_2Cl_2, rt, quant. (two steps); (c) LiDBB, THF, −78 °C, 90%; (d) SO_3·pyridine, Et_3N, CH_2Cl_2/DMSO, rt; (e) $Ph_3P{=}C(Me)COOEt$, CH_2Cl_2, rt, 80% (two steps); (f) DIBALH, CH_2Cl_2, −78 °C, 98%; (g) TASF, THF/DMF, rt, 70%; (h) MnO_2, THF/$CHCl_3$, rt, 74%.

deprotection, followed by chemoselective oxidation, furnished analogue **7**. Analogue **8** was synthesized in a similar way.

To examine the molecular length of gymnocin-A required for exhibiting cytotoxicity, we next synthesized the truncated analogues **9** and **10** having a left-hand structure and 2-methyl-2-butenal side chain. The synthesis of analogue **9** commenced with alcohol **16**,[8b,d] which was converted to **17** by a three-step sequence of protective group manipulations (Scheme 2). Subsequent oxidation and Wittig reaction afforded α,β-unsaturated ester **18**. DIBALH reduction followed by desilylation with TASF led to allylic alcohol **19**, which upon oxidation with MnO_2 furnished the desired truncated analogue **9**.

The synthesis of the other truncated analogue **10** started with the Suzuki–Miyaura coupling reaction of an alkylborane, generated from exocyclic enol ether **20**, with enol triflate **21**[13] (Scheme 3). The resultant cross-coupled product **22** was further converted to ketone **23** by a four-step sequence of reactions. In a similar way, in the total synthesis of gymnocin-A,[8c,d] ketone **23** was elaborated to nonacyclic polyether **27** through stereoselective introduction of the C10 hydroxy group (**23** → **24**), formation of the E-ring as a mixed thioacetal



Scheme 3. Reagents and conditions: (a) **20**, 9-BBN, THF, rt; then aq Cs_2CO_3, $Pd(PPh_3)_4$, DMF, rt; (b) BH_3·SMe_2, THF, 0 °C → rt; then aq NaOH, H_2O_2, rt, 54% (two steps); (c) TESOTf, 2,6-lutidine, CH_2Cl_2, rt; (d) DDQ, CH_2Cl_2/pH 7 buffer, rt, 83% (two steps); (e) TPAP, NMO, 4 Å MS, CH_2Cl_2, rt, 97%; (f) LiHMDS, TMSCl, Et_3N, THF, −78 °C; (g) OsO_4, NMO, THF/H_2O, rt, 93% (two steps); (h) TIPSOTf, 2,6-lutidine, CH_2Cl_2, rt, 88%; (i) EtSH, $Zn(OTf)_2$, CH_2Cl_2/CH_3NO_2, 0 °C, **25**, 20%; **26**, 73%; (j) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt, 84%; (k) Ph_3SnH, AIBN, toluene, 110 °C, 77%; (l) TBAF, 4 Å MS, THF/CH_3CN, 70 °C; (m) TESOTf, 2,6-lutidine, CH_2Cl_2, rt; (n) LiDBB, THF, −78 °C, 79% (three steps); (o) TPAP, NMO, 4 Å MS, CH_2Cl_2, rt; (p) $Ph_3P{=}C(Me)COOEt$, CH_2Cl_2, rt; (q) DIBALH, CH_2Cl_2, −78 °C; (r) TASF, THF/DMF, rt, 49% (four steps); (s) MnO_2, THF/$CHCl_3$, rt, 93%.

Table 1. Cytotoxicity of gymnocin-A (**1**) and synthetic analogues (**5**–**10**, **19**, and **29**) against P388 murine leukemia cells

Compounds	Cytotoxicity IC_{50}, μM
1	1.3
5	>100
6	>10
7	1.0
8	2.9
9	>100
10	>100
19	>100
29	>100

(**24** → **26**), and reductive desulfurization under radical conditions[14] (**26** → **27**). Protective group manipulations of **27** led to primary alcohol **28**, which was then converted to allylic alcohol **29** by a four-step sequence. Finally, oxidation of allylic alcohol **29** with MnO_2 led to truncated analogue **10**.

With gymnocin-A analogues **5**–**10**, **19**, and **29** in hand, the biological activity of these compounds was evaluated by their inhibitory activity against the murine leukemia cell line (P388D1) using the XTT assay[15] and the results are summarized in Table 1. α,β-Unsaturated aldehydes **7** and **8** exhibited cytotoxicity with IC_{50} values of 1–3 μM, comparable to that of the natural gymnocin-A, whereas α,β-unsaturated ester **5** and carboxylic acid **6** did not show cytotoxicity even at a concentration of 100 μM. These results clearly indicate that the α,β-unsaturated aldehyde functionality is crucial for its cytotoxicity. This is probably due to nucleophilic addition of biological macromolecules to a reactive electrophilic center. In addition, nonacyclic analogue **10** somewhat decreased proliferation of P388 cells at 100 μM, whereas truncated analogues **9**, **19**, and **29** showed no detectable cytotoxicity even at 100 μM. Consequently, the molecular length was also important for exhibiting cytotoxicity.

In summary, the present structure–activity relationship studies revealed that the structural elements required for cytotoxicity of gymnocin-A are not only the α,β-unsaturated aldehyde functionality of the side chain but also the molecular length, probably over ten contiguous trans-fused rings. These studies would help to understand the relation between the common polycyclic ether motif and diverse biological activities.

Acknowledgments

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