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Synthesis and Biological Evaluation of Gambierol Analogues

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Abstract—Gambierol is a polycyclic ether toxin, isolated as a toxic constituent from the marine dinoflagellate *Gambierdiscus toxicus*. We describe here the synthesis and biological evaluation of structural analogues of gambierol. The present preliminary structure–activity relationship studies clearly indicate that the H ring functionality and the unsaturated side chain of gambierol are crucial for its potent toxicity.

The polycyclic ether class of marine natural products, exemplified by brevetoxins, ciguatoxins and maitotoxin, has received much attention due to the diverse biological activities with extreme potency and their complex molecular architecture.[1] Among the most prominent examples are ciguatoxin and its congeners.[2,3] These are the principal toxins for ciguatera fish poisoning that is prevalent in circumtropical areas with more than 20,000 victims annually. The causative toxins originate in an epiphytic dinoflagellate, *Gambierdiscus toxicus,* and are accumulated in fish through the food chain, thus causing human intoxication.[4] The ciguatoxins are extremely potent neurotoxins that bind to voltage-sensitive sodium channels and alter their function.[5]

Gambierol (**1**) is another polycyclic ether toxin isolated with ciguatoxin congeners from the culture cells of *G. toxicus* collected at the Rangiroa Peninsula in French Polynesia.[6,7] It exhibits potent lethality against mice at 50 μg/kg (ip), and its neurological symptoms caused in mice resemble those shown by ciguatoxins, implying the possibility that gambierol is also responsible for ciguatera fish poisoning. However, the extremely limited availability of this toxin from natural sources has hampered detailed biological studies.[8] Therefore, supply of useful quantities of this natural product by chemical synthesis is strongly demanded and a number of synthetic efforts have been reported to date,[9] culminating in completed total synthesis by us[10] and the Yamamoto group.[11] The convergent and flexible entry to gambierol by our total synthesis[10] has since allowed the generation of a series of analogues to elucidate the structure–activity relationship (SAR). We report here the synthesis of gambierol analogues with modifications in the H ring and the side chain and evaluation of their toxicity against mice. The described preliminary SAR studies, which were made possible only through chemical synthesis, provided the first insight into the critical structural elements for the potent lethality of gambierol (Fig. 1).

As a first step of the SAR study of gambierol, we focused on modifications of the right-hand portion of the molecule, namely, the H ring functionality and the lipophilic triene side chain. Initially, we prepared compounds **3** and **4** in order to investigate whether the polyether core of **1** alone is sufficient for exerting toxicity. Removal of the benzyl groups of **2**[9k,10] by hydrogenolysis provided diol **3**, which was then deacetylated to give tetraol **4** (Scheme 1).

Next, analogues **9**, **13**, and **16**, lacking the C30[12] methyl group, were synthesized. Enone **5**[10] was subjected to

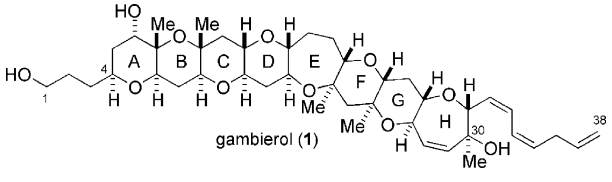

Figure 1. Structure of gambierol (**1**).

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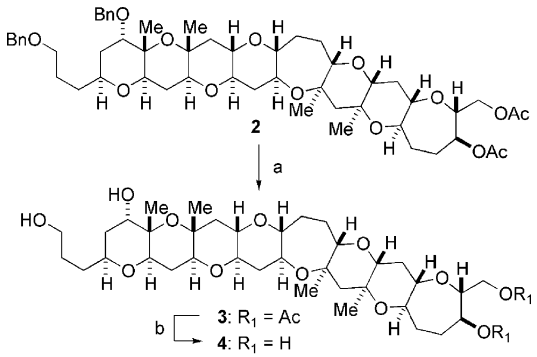
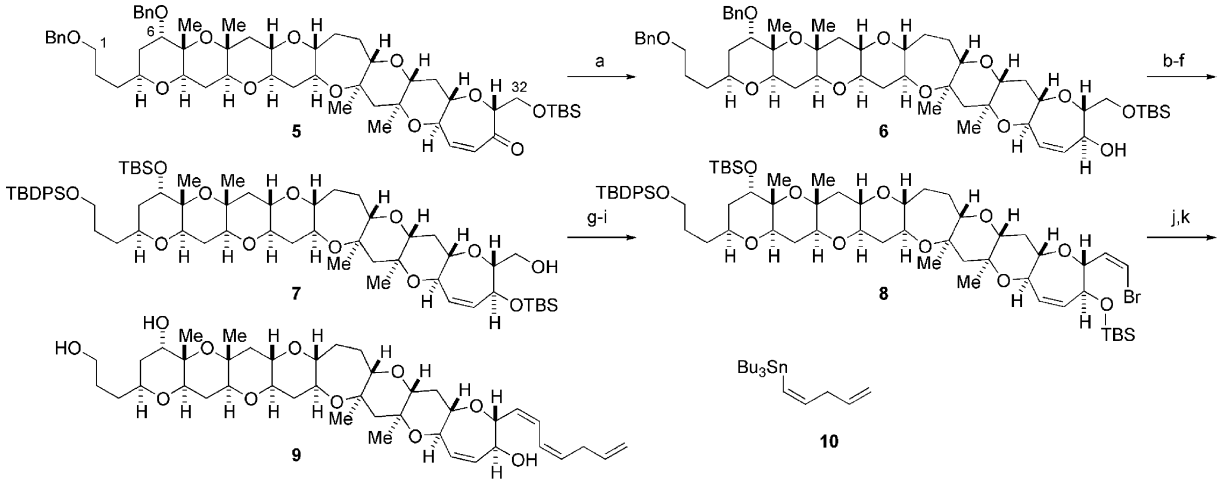


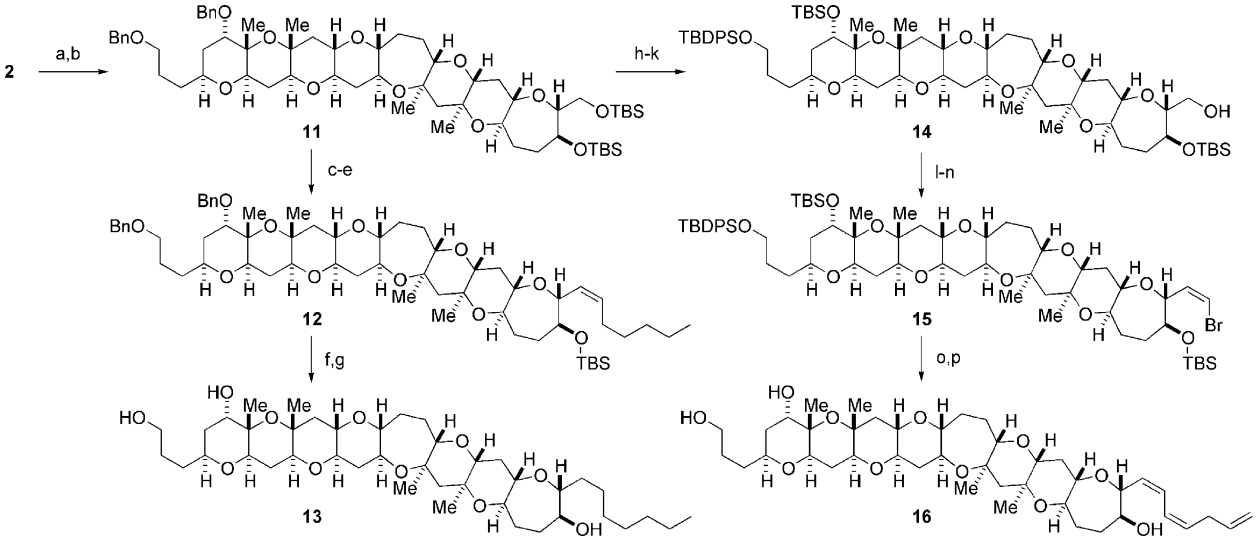
Scheme 1. Reagents and conditions: (a) H_2, $Pd(OH)_2/C$, EtOAc, rt, 94%; (b) K_2CO_3, MeOH, rt, 84%.

Luche reduction[13] to give stereoselectively allylic alcohol **6** (78% yield), which was then converted to alcohol **7** by a five-step sequence of protective group manipulations (Scheme 2). The strategy used in the total synthesis of **1** allowed for conversion of **7** into compound **9** via (*Z*)-vinyl bromide **8**.[10]

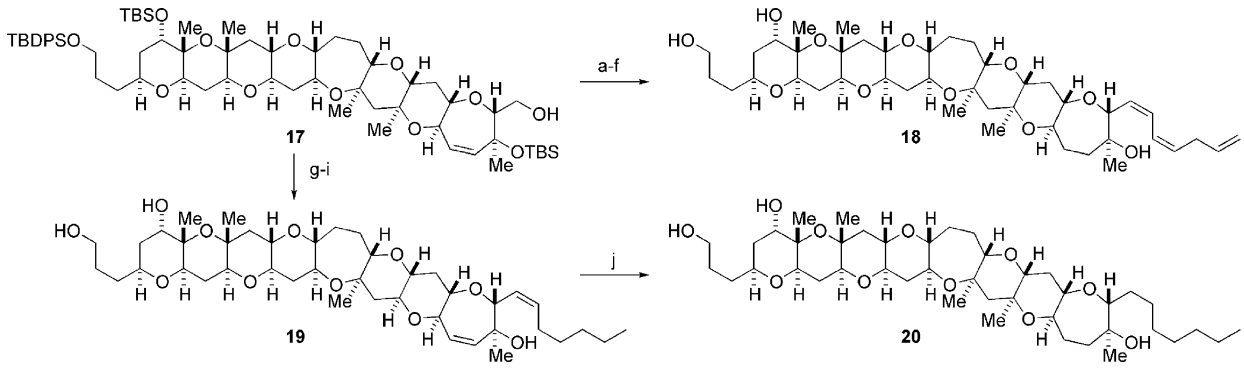
Syntheses of compounds **13** and **16** commenced with replacement of the acetyl groups of **2**[9k,10] with *tert*-butyldimethylsilyl (TBS) groups to give bis(silyl) ether **11** in high yield (Scheme 3). Selective liberation of the C32 primary hydroxyl group followed by oxidation and Wittig reaction of the derived aldehyde led to (*Z*)-olefin **12**. Removal of the TBS ether and hydrogenolysis of the benzyl ethers with concomitant reduction of the



Scheme 2. Reagents and conditions: (a) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH/CH_2Cl_2 (1:1), 0 °C, 78%; (b) TBSOTf, Et_3N, CH_2Cl_2, 0 °C; (c) LiDBB, THF, −78→−40 °C, 75% (two steps); (d) TBDPSCl, Et_3N, DMAP, CH_2Cl_2, rt, 85%; (e) TBSOTf, Et_3N, CH_2Cl_2, rt; (f) CSA, MeOH/CH_2Cl_2 (1:1), 0 °C, 81% (two steps); (g) SO_3·pyr, Et_3N, DMSO/CH_2Cl_2 (1:1), 0 °C; (h) CBr_4, PPh_3, Et_3N, CH_2Cl_2, 0 °C, 47% (two steps); (i) *n*-Bu_3SnH, $Pd(PPh_3)_4$, benzene, rt; (j) HF·pyr, THF, rt; (k) **10**, $Pd(PPh_3)_4$, CuCl, LiCl, DMSO/THF (1:1), 60 °C, 34% (three steps).



Scheme 3. Reagents and conditions: (a) K_2CO_3, MeOH, rt; (b) TBSOTf, Et_3N, DMF, 0 °C, 99% (two steps); (c) CSA, MeOH/CH_2Cl_2 (1:1), 0 °C, 59%; (d) TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, rt; (e) $CH_3(CH_2)_5P^+Ph_3Br^-$, NaHMDS, THF, 0 °C, 45% (two steps); (f) HF·pyr, THF, rt, 72%; (g) H_2, $Pd(OH)_2/C$, MeOH/EtOAc (1:1), rt, quant; (h) LiDBB, THF, −78→−40 °C; (i) TBDPSCl, Et_3N, DMAP, CH_2Cl_2, rt, 79% (two steps); (j) TBSOTf, Et_3N, CH_2Cl_2, 0 °C→rt; (k) CSA, MeOH/CH_2Cl_2 (1:1), 0 °C, 80% (two steps); (l) SO_3·pyr, Et_3N, DMSO/CH_2Cl_2 (1:1), 0 °C; (m) CBr_4, PPh_3, Et_3N, CH_2Cl_2, 0 °C, 92% (two steps); (n) *n*-Bu_3SnH, $Pd(PPh_3)_4$, benzene, rt, 47%; (o) HF·pyr, THF, rt, 97%; (p) **10**, $Pd(PPh_3)_4$, CuCl, LiCl, DMSO/THF (1:1), 60 °C, 68%.



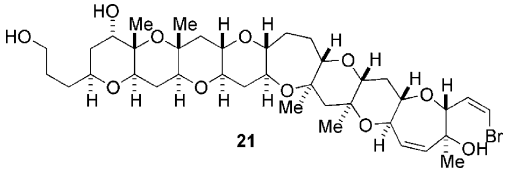
Scheme 4. Reagents and conditions: (a) H_2, Pd/C, EtOAc, rt, 95%; (b) SO_3·pyr, Et_3N, DMSO/CH_2Cl_2 (1:1), 0 °C; (c) CBr_4, PPh_3, Et_3N, CH_2Cl_2, 0 °C; (d) *n*-Bu_3SnH, $Pd(PPh_3)_4$, benzene, rt, 74% (three steps); (e) HF·pyr, THF, rt, quant; (f) **10**, $Pd(PPh_3)_4$, CuCl, LiCl, DMSO/THF (1:1), 60 °C, 64%; (g) SO_3·pyr, Et_3N, DMSO/CH_2Cl_2 (1:1), 0 °C; (h) $CH_3(CH_2)_5P^+Ph_3Br^-$, NaHMDS, THF, 0 °C, 58% (two steps); (i) HF·pyr, THF, rt, 81%; (j) H_2, Pd/C, EtOAc, rt, 59%.

C32–C33 double bond then afforded compound **13**. On the other hand, **11** was converted into alcohol **14** by a four-step sequence of reactions including reductive debenzylation with lithium di-*tert*-butylbiphenilide (LiDBB), two-step protection of the C1 and C6 hydroxyl groups, and selective liberation of the C32 hydroxyl group under acidic conditions. Conversion of **14** into compound **16** via **15** followed the same protocol as described earlier.

Syntheses of analogues **18**–**20**, having the axial C30 methyl group, are summarized in Scheme 4. Conversion of **17**[10] into compound **18** was carried out following the protocol used for the synthesis of **9** and **16**, while compound **19** was prepared from **17** in a similar way used for the synthesis of **12**. Finally, hydorogenation of **19** gave compound **20**.

With the gambierol analogues (**3**, **4**, **9**, **13**, **16**, and **18**–**20**) in hand, the respective toxicities against male mice (ddY strain, 12–17 g body weight) were determined by intraperitoneal injection of suspensions in 1% Tween 60 at appropriate dose levels, along with the parent synthetic **1** and analogue **21**[10] with truncated side chain (Table 1).[14] Not unexpectedly, compounds **3** and **4** were completely inactive, indicating that the octacyclic polyether core alone is not sufficient for exhibiting toxicity. Compound **9** lacking the axial C30 methyl group still retained a significant level of lethality, being approximately 5-fold less active than the parent **1**. Therefore, the C30 methyl group is not critical for exerting toxicity, while possibly important. Compound **19**, 34,35,37,38-tetrahydro derivative of **1**, exhibited toxicity to a lower extent, which is approximately 25-fold lower than that of **1**, whereas compound **18**, in which the C28–C29 double bond is removed, suffered a further decrease in its toxicity (ca. 120-fold less). Mice died within 15–30 min after ip injection of these three active compounds. In contrast, compounds **13**, **16**, **20**, and **21** showed no detectable toxicity against mice even at >7.6 mg/kg dose level.[15] These results clearly demonstrate that the H ring double bond and the side chain containing the C28–C29 (*Z*)-double bond are critical structural requirements for potent lethality of **1**. These structural elements would serve to orientate the side chain to the proper direction.[16] Moreover, the proper length of the side chain also seems to be necessary for toxicity because truncating the side chain (compound **21**) resulted in loss of activity.

Table 1. Minimal lethal dose values (mg/kg) of compounds **1**, **3**, **4**, **9**, **13**, **16** and **18**–**21** in mice



Compd	Minimal lethal dose (mg/kg)
1	0.05–0.07
3	>8.2
4	>18.3
9	0.34
12	>7.6
16	>11.9
18	7.9
19	1.6
20	>12.9
21	>7.6

In summary, the present SAR studies indicate that the structural elements required for potent toxicity of **1** are not only the fused polycyclic ether core structure but also the H ring double bond as well as the side chain with an appropriate length and a double bond between C32 and C33. Although the analogues we have prepared are inactive or less potent than **1**, these synthetic analogues cannot be prepared by derivatization from natural **1**. On the basis of the present studies, further efforts aimed at understanding the molecular basis of the potent lethality of gambierol are currently underway and will be reported in due course.

Acknowledgements

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14. All samples used in the assay were purified by HPLC (Asahipak ODP-506D, ϕ 4.6×150 mm) using CH_3CN/H_2O as an eluent.

15. For compounds **18** and **19**, upon ip injection at lower dose levels typical neurological symptoms were observed in mice, whereas such changes were not observed for other inactive compounds.

16. Details of conformational analysis of analogues will be reported in a full account.