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Total Synthesis and Complete Stereostructure of Gambieric Acid A

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Supporting Information

ABSTRACT: Total synthesis of gambieric acid A, a potent antifungal polycyclic ether metabolite, has been accomplished for the first time, which firmly established the complete stereostructure of this natural product.

Marine polycyclic ether natural products constitute one of the most intriguing families of secondary metabolites known to date.[1,2] This intrigue is due primarily to their highly complex molecular architecture, potent biological activities, and extreme natural scarcity. Gambieric acids (GAs) A–D (Figure 1) were isolated from the cultured cells of the ciguatera causative dinoflagellate *Gambierdiscus toxicus* (GII1 strain) by Nagai, Yasumoto, and co-workers.[3] The gross structure, including the relative stereochemistry of the polycyclic ether domain, was determined through extensive 2D NMR spectroscopic analyses. The complete stereostructure of GAs was assigned on the basis of NMR-based conformational analysis, degradation experiments, the application of chiral anisotropic reagents, and chiral HPLC analysis.[4] However, our own studies on model compounds have suggested that the absolute configuration of the polycyclic ether domain of GAs is opposite to that of the originally assigned structure.[5] Accordingly, we proposed the stereostructure of GAs shown in Figure 1; however, this structure needed to be confirmed by total synthesis. (+)-Gambieric acid A (**1**) exhibits exceedingly potent antifungal activity. Specifically, **1** is approximately 2000 times more potent than amphotericin B. It is known that **1** only weakly displaces tritiated dihydrobrevetoxin-B ([^{3}H]PbTx-3) from voltage-gated sodium ion channels[6] and does not show any detectable toxicity against mice at 1 mg/kg, which was the highest dose tested,[7] even though **1** is structurally similar to ion channel-activating polycyclic ether neurotoxins, such as brevetoxins and ciguatoxins.[1] Meanwhile, Nagai et al. suggested that **1** has a possible role as an endogenous growth-regulating factor of *G. toxicus*.[8] However, detailed investigations into the biological mode-of-actions of GAs have been precluded by their limited availability from natural sources. Although recent methodological advances[2] have greatly improved our ability to synthesize complex polycyclic ether molecules, GAs remain unconquered because of their anomalous structural complexity, which poses a formidable synthetic challenge to organic chemists.[9,10] Here, we disclose the first total synthesis of **1**, which resulted in unambiguous establishment of its complete stereostructure.

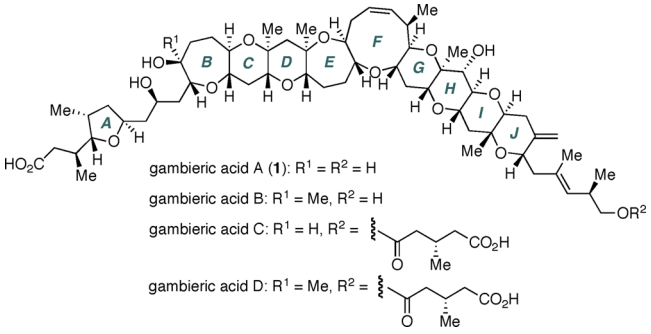
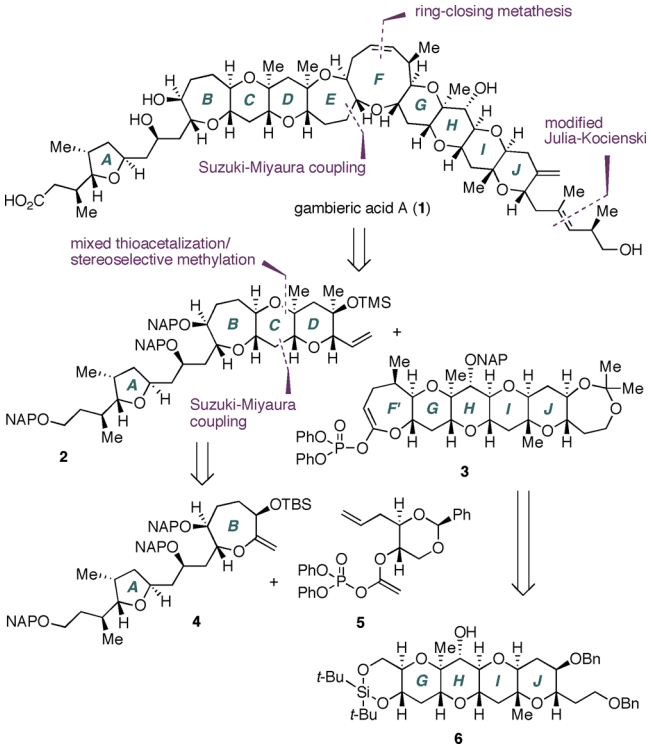


Figure 1. Structures of gambieric acids A–D.

Our synthetic plan toward **1** is illustrated in Scheme 1. We planned to introduce the J-ring side chain at a late stage of the total synthesis, by using the modified Julia–Kocienski olefination.[10g,h,11] We retrosynthetically divided the nonacyclic core structure of **1** into the A/BCD-ring fragment **2** and F′GHIJ-ring fragment **3** containing a contracted F′-ring moiety. These two intermediates of equal structural complexity would

Scheme 1. Synthesis Plan toward 1



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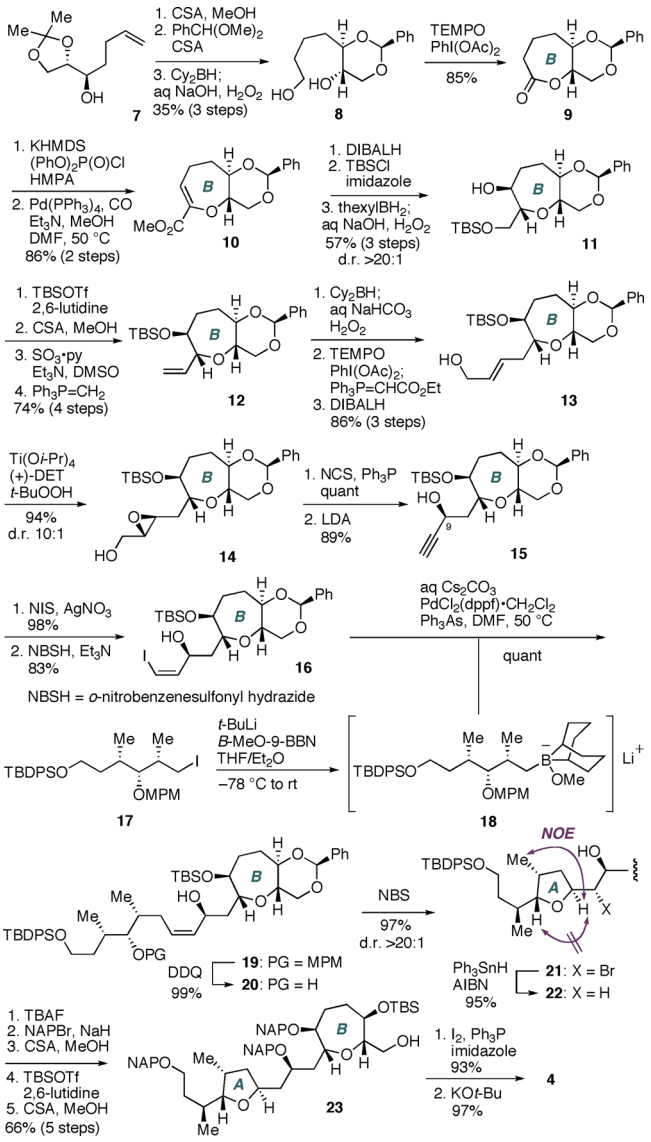
be assembled through a Suzuki–Miyaura coupling,[12,13] and the successive formation of the E- and F-rings would complete the nonacyclic core of **1** with simultaneous control of the stereogenic centers present around the central domain of the molecule. We envisioned that **2** would be available from the A/B-ring *exo*-olefin **4** and enol phosphate **5** through a Suzuki–Miyaura coupling and ring-closing metathesis (RCM),[14,15] followed by closure of the C-ring via the mixed thioacetalization methodology.[16] Meanwhile, the F′GHIJ-ring fragment **3** would be prepared from our previously reported tetracyclic ether **6**,[10g,h] which represents the GHIJ-ring.

The synthesis of the A/B-ring fragment **4** commenced with known alcohol **7**,[17] which was converted to diol **8** in three steps (Scheme 2). The oxidative lactonization[18] of **8** proceeded

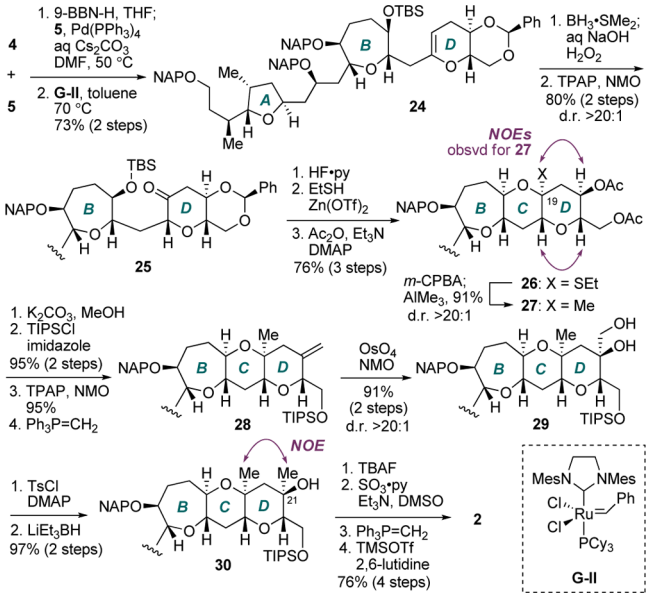
Scheme 2. Synthesis of the A/B-ring fragment 4



smoothly to provide seven-membered lactone **9**, which was converted to α,β-unsaturated ester **10** via palladium-catalyzed methoxycarbonylation of the corresponding enol phosphate.[19] Reduction of **10**, followed by silylation, and subsequent stereoselective hydroboration provided alcohol **11** as a single stereoisomer. Alcohol **11** was transformed to allylic alcohol **13** by standard chemistry. Sharpless asymmetric epoxidation provided epoxy alcohol **14** with 10:1 diastereoselectivity. The chlorination of **14**, followed by treatment with lithium diisopropylamide (LDA),[20] afforded propargylic alcohol **15**. At this point, the minor C9 diastereomer could be removed by flash chromatography using silica gel. The iodination of the terminal alkyne and diimide reduction[21] of the resultant iodoalkyne led to (*Z*)-vinyl iodide **16**. Iodide **16** was coupled with alkylborate **18**, which was first generated *in situ* from iodide **17**,[22] by means of a Suzuki–Miyaura coupling to afford *cis*-olefin **19** quantiatively. After removing the *p*-methoxyphenylmethyl (MPM) group, the resultant olefin **20** was treated with NBS to promote stereoselective bromoetherification to deliver bromide **21** in 97% yield as a single stereoisomer. Subsequent reductive removal of the bromine atom gave tetrahydrofuran derivative **22**. At this point, the relative stereochemistry of the A-ring tetrahydrofuran was established using the observed NOE. Subsequent routine protective group manipulations led to alcohol **23**, which was converted to *exo*-olefin **4** via iodination and base treatment.

With the A/B-ring *exo*-olefin **4** in hand, we proceeded to construct the C- and D-rings using the methodologies developed in our laboratory (Scheme 3). The Suzuki–Miyaura

Scheme 3. Synthesis of the A/BCD-ring Fragment 2



coupling of an alkylborane derived from **4** with acetate-derived enol phosphate **5**[22] afforded an acyclic enol ether, which was immediately subjected to RCM using the Grubbs second-generation catalyst (**G-II**).[23] This two-step sequence[15] provided endocyclic enol ether **24** in good overall yield. Stereoselective hydroboration of **24**, followed by oxidation of the resultant alcohol, gave ketone **25** as a single stereoisomer. After the removal of the silyl group, the resultant hemiacetal was exposed to EtSH in the presence of zinc trifluoromethanesulfonate [$Zn(OTf)_2$][16a] to promote mixed thioacetalization and concomitant cleavage of the benzylidene acetal. Subsequent acetylation of the liberated hydroxy groups gave mixed thioacetal **26**. Stereoselective introduction of the C19 methyl group using a one-pot oxidation/methylation protocol[16b] afforded tetracyclic ether **27** as a single stereoisomer. The relative stereochemistry surrounding the C-ring was established

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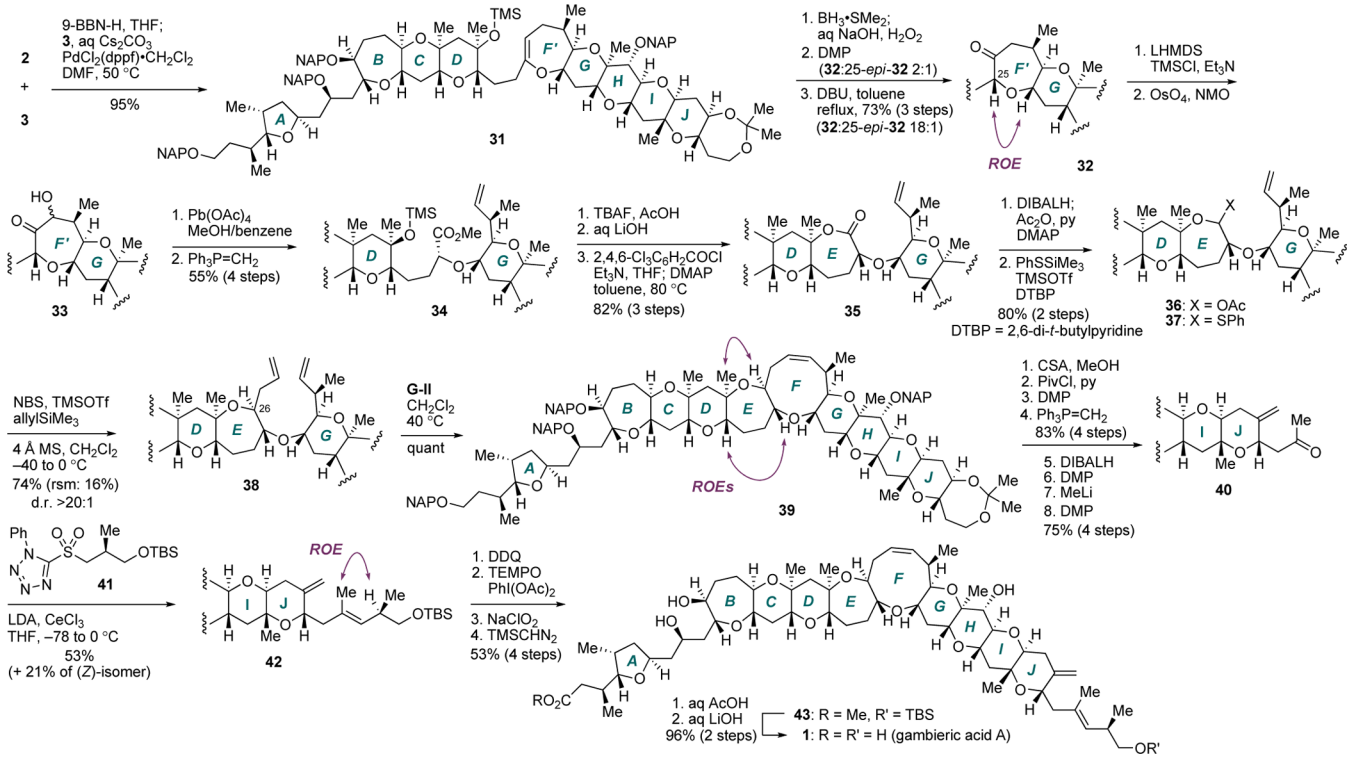
Scheme 4. Completion of the Total Synthesis of 1



on the basis of NOE experiments as shown. To complete the A/BCD-ring fragment, the C21 axial methyl group had to be introduced onto the D-ring. To this end, **27** was elaborated to *exo*-olefin **28** in four steps. Dihydroxylation of **28** proceeded from the sterically less encumbered face of the molecule to give 1,2-diol **29** as a single stereoisomer.[16a] Tosylation of **29** and subsequent $LiEt_3BH$ reduction uneventfully afforded tertiary alcohol **30**. Finally, desilylation, oxidation, methylenation, and silylation furnished the A/BCD-ring fragment **2**.

The completion of the total synthesis of **1** is illustrated in Scheme 4. First, we assembled the A/BCD- and F′GHIJ-ring fragments (**2** and **3**,[22] respectively) through a Suzuki–Miyaura coupling, which afforded endocyclic enol ether **31** in a respectable yield. Hydroboration of **31** delivered a 2:1 mixture of diastereomeric alcohols. Without separation, these alcohols were oxidized to give an epimeric mixture of ketones **32** and 25-*epi*-**32** (not shown), which was then treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to promote epimerization at the C25 stereogenic center. This allowed us to convert the most of the unwanted 25-*epi*-**32** into the desired **32**, the latter being thermodynamically favored under equilibrating conditions (**32**:25-*epi*-**32** = 18:1, after epimerization). The C25 stereochemistry was confirmed by an ROE experiment, as shown. Having successfully established the C25 stereogenic center, the F′-ring was oxidatively cleaved via the intermediacy of α-hydroxy ketone **33**. Thus, dihydroxylation of the silyl enol ether derived from **32** gave **33**, which was exposed to $Pb(OAc)_4$ in methanol/benzene, and then immediately methylenated to deliver ester **34**. Desilylation, followed by saponification of the methyl ester, and ensuing lactonization[24] afforded lactone **35**. Reductive acetylation[25] of **35** gave α-acetoxy ether **36**. Our previous studies on model compounds suggested that harsh reaction conditions would be necessary for direct allylation of **36** due to its low reactivity.[10a–c,e,f] Instead, we envisaged an application of our glycosylation chemistry[26] to the corresponding mixed thioacetal **37**. Thus, stereoselective allylation to complete the E-ring was best accomplished by exposing **37** to allyltrimethylsilane in the presence of NBS/trimethylsilyl trifluoromethanesulfonate (TMSOTf) to provide diene **38** in 74% yield as a single stereoisomer with correct C26 stereochemistry. RCM of **38** using **G-II** cleanly furnished nonacyclic core **39** quantitatively. The relative configurations of the stereogenic centers around the E- and F-rings were confirmed at this stage by the ROE experiment as shown.

The J-ring side chain needed to be constructed to complete the total synthesis. Toward this end, **39** was converted to methyl ketone **40** via an eight-step sequence. Julia–Kocienski olefination of **40** using sulfone **41**[10g,h] was performed in the presence of $CeCl_3$[10g,h] to afford the desired trisubstituted olefin **42** together with the corresponding (*Z*)-isomer (not shown). These isomers were separated by flash chromatography using silica gel. Oxidative cleavage of the 2-naphthylmethyl (NAP) ethers, selective two-stage oxidation of the liberated primary hydroxy group, and subsequent esterification provided methyl ester **43**. Finally, acidic cleavage of the silyl ether and saponification of the methyl ester furnished (+)-gambieric acid A (**1**). The spectroscopic properties (IR, ^1H, ^{13}C NMR, and HRMS) and optical rotation value of synthetic (+)-**1** ($[\alpha]_D^{25}$ +22.5 (*c* 0.40, MeOH)) were in full accordance with those of the authentic sample ($[\alpha]_D^{20}$ +33 (*c* 0.488, MeOH)).[3] Furthermore, the synthetic material displayed antifungal activity against *Aspergillus niger*, which was equipotent to that of the natural product. Thus, we conclude that the structure **1**, shown in Figure 1, represents the complete stereostructure of (+)-gambieric acid A, confirming our previous stereochemical reassignment on the basis of the synthesis and spectroscopic analysis of model compounds.[5]

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In conclusion, we have completed the total synthesis of (+)-gambieric acid A (**1**) for the first time by exploiting synthetic methodologies developed within our laboratory. The chemistry described herein opens up avenue for the preparation of synthetic analogues that would be helpful for addressing the biological profile of **1**, which is uniquely different from that of ion channel-activating polycyclic ether neurotoxins despite the high structural similarity shared among this class of natural products. This study also demonstrated the vital role of total synthesis in the structure determination of complex natural products.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and spectroscopic data for all new compounds and copies of ^{1}H and ^{13}C NMR spectra for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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