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Studies toward the Total Synthesis of Gambieric Acids: Stereocontrolled Synthesis of a DEFG-Ring Model Compound

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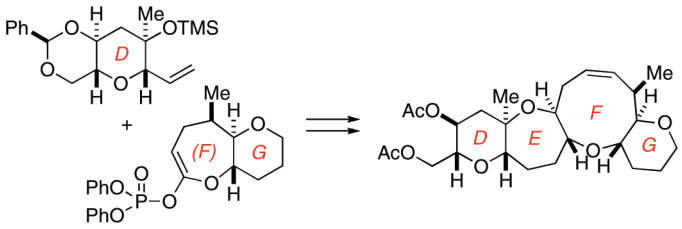

A stereocontrolled convergent synthesis of a DEFG-ring model compound of gambieric acids, highly potent antifungal marine polycyclic ether natural products, has been achieved based on Suzuki—Miyaura coupling. Conformational analysis of the model compound revealed that the nine-membered F-ring exists exclusively as a single stable conformer, as opposed to that of ciguatoxins.

Introduction

Gambieric acids (GAs), isolated from a strain of the ciguatera causative dinoflagellate *Gambierdiscus toxicus* by Yasumoto and co-workers, are structurally characterized by the nonacyclic polyether core structure decorated with a complex side chain containing a 2,5-*trans*-substituted tetrahydrofuran ring (Figure 1).[1] The gross structure and partial relative stereochemistry of gambieric acids was determined by extensive 2D-NMR studies, and the complete stereostructure was subsequently proposed on the basis of degradation experiments, application of the modified Mosher analysis, and chiral HPLC analysis.[2] Recently, chemical synthesis and NMR analysis of the A/B- and A/BC-ring model compounds of GAs in our group strongly suggested that the absolute configuration of the nonacyclic polyether core is opposite to that of the proposed structure, as shown in Figure 1.[3] Gambieric acid A (GAA), the representative congener, displays extremely potent antifungal activity (approximately 2,000 times more potent than amphotericin B) with minimal toxicity against mice or cultured mammalian cells,[4] although the biochemical mode of action remains to be unraveled because of the lack of material supply from natural sources. GAA is also known to displace tritium-labeled dihydrobrevetoxin-B ([^{3}H]PbTx-3) bound to voltage-sensitive sodium ion channels (VSSCs) in micromolar concentrations.[5] Nagai and co-workers reported that GAA acts as an endogenous growth-regulating factor of *G. toxicus*.[6] These intriguing structural and biological aspects of GAs gained significant interest from synthetic organic chemists.[7]

Herein, we report in detail a stereocontrolled convergent synthesis of the DEFG-ring skeleton **1** of GAs (Scheme 1).[8] Our first-generation approach utilized Horner—Wadsworth—Emmons (HWE) coupling as a key fragment assembly

(1) (a) Nagai, H.; Torigoe, K.; Satake, M.; Murata, M.; Yasumoto, T.; Hirota, H. *J. Am. Chem. Soc.* **1992**, *114*, 1102–1103. (b) Nagai, H.; Murata, M.; Torigoe, K.; Satake, M.; Yasumoto, T. *J. Org. Chem.* **1992**, *57*, 5448–5453.

(2) Morohashi, A.; Satake, M.; Nagai, H.; Oshima, Y.; Yasumoto, T. *Tetrahedron* **2000**, *56*, 8995–9001.

(3) (a) Fuwa, H.; Ishigai, K.; Goto, T.; Suzuki, A.; Sasaki, M. *J. Org. Chem.* **2009**, *74*, 4024–4040. (b) Fuwa, H.; Goto, T.; Sasaki, M. *Org. Lett.* **2008**, *10*, 2211–2214. (c) Fuwa, H.; Suzuki, A.; Sato, K.; Sasaki, M. *Heterocycles* **2007**, *72*, 139–144.

(4) Nagai, H.; Mikami, Y.; Yazawa, K.; Gonoi, T.; Yasumoto, T. *J. Antibiot.* **1993**, *46*, 520–522.

(5) Inoue, M.; Hirama, M.; Satake, M.; Sugiyama, K.; Yasumoto, T. *Toxicon* **2003**, *41*, 469–474.

(6) Sakamoto, B.; Nagai, H.; Yasumoto, T. *Phycologia* **1996**, *35*, 350–353.

(7) (a) Kadota, I.; Oguro, N.; Yamamoto, Y. *Tetrahedron Lett.* **2001**, *42*, 3645–3647. (b) Kadota, I.; Takamura, H.; Yamamoto, Y. *Tetrahedron Lett.* **2001**, *42*, 3649–3651. (c) Clark, J. S.; Fessard, T. C.; Wilson, C. *Org. Lett.* **2004**, *6*, 1773–1777. (d) Clark, J. S.; Kimber, M. C.; Robertson, J.; McErlean, C. S. P.; Wilson, C. *Angew. Chem., Int. Ed.* **2005**, *44*, 6157–6162. (e) Sato, K.; Sasaki, M. *Org. Lett.* **2005**, *7*, 2441–2444. (f) Roberts, S. W.; Rainier, J. D. *Org. Lett.* **2007**, *9*, 2227–2230. (g) Sato, K.; Sasaki, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2518–2522. (h) Sato, K.; Sasaki, M. *Tetrahedron* **2007**, *63*, 5977–6003. (i) Saito, T.; Nakata, T. *Org. Lett.* **2009**, *11*, 113–116.

(8) For a preliminary communication, see: Fuwa, H.; Noji, S.; Sasaki, M. *Chem. Lett.* **2009**, *38*, 866–867.

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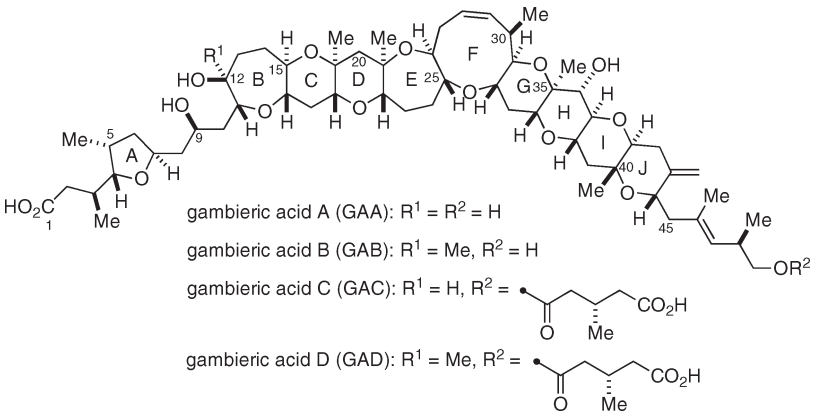
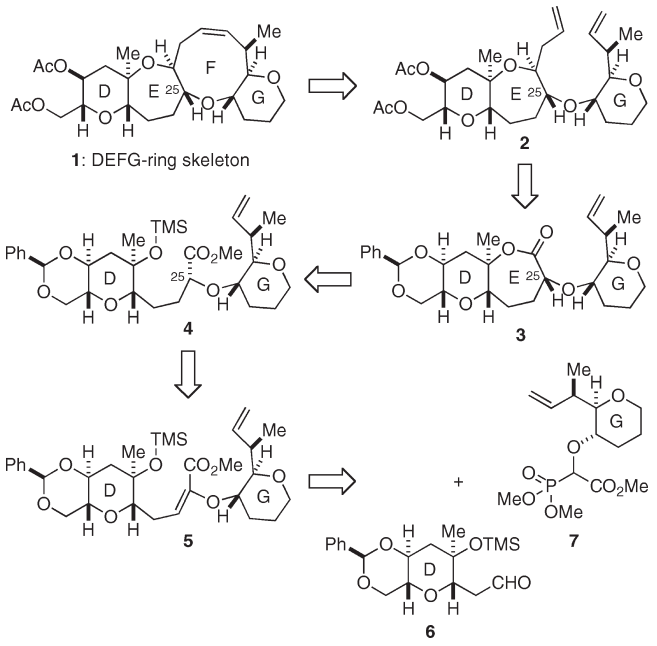


FIGURE 1. Structures of gambieric acids A—D.

SCHEME 1. First-Generation Synthesis Plan

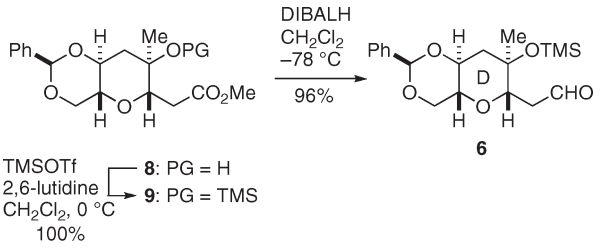


process, but this approach eventually proved to be unrewarding because of the difficulty in controlling the stereochemistry of the C25 stereogenic center. The second-generation approach relied on Suzuki—Miyaura coupling[9] for the convergent union of the D- and G-ring fragments. The C25 stereogenic center was successfully defined by making use of the intrinsic substrate bias of seven-membered cyclic ethers.

Results and Discussion

First-Generation Synthesis Plan toward the DEFG-Ring Skeleton. As already described in previous reports from this laboratory, ring-closing metathesis (RCM)[10] was considered

SCHEME 2. Synthesis of Aldehyde 6



to be a suitable means to construct the F-ring of **1**, and diene **2** would be available from ester **4** via lactone **3** (Scheme 1). The major challenge in the synthesis of **4** is the stereoselective construction of the C25 stereogenic center. Indeed, the lack of stereocontrol at C25 was a serious drawback in our previous synthesis of the nonacyclic skeleton of GAs.[7g,h] We envisaged that the desired ester **4** would be derived from enoate **5** via stereoselective 1,4-reduction, and the latter could be dissected to the D-ring aldehyde **6** and the G-ring phosphonate **7** through a HWE reaction.

Synthesis of the D-Ring Fragment 6. The synthesis of the D-ring fragment **6** is summarized in Scheme 2. The known ester **8**[11] was protected as its TMS ether **9**. DIBALH reduction of **9** then gave aldehyde **6**.

Synthesis of the G-Ring Phosphonate 7. We started the synthesis of the G-ring fragment **7** from the known alcohol **10**[12] (Scheme 3). A three-step sequence that involved Parihk—Doering oxidation,[13] Grignard reaction, and PCC oxidation gave methyl ketone **11**. Wittig methylenation of **11** with $Ph_3P{=}CH_2$ gave olefin **12**. Stereoselective hydroboration of **12** using dicyclohexylborane in THF at room temperature followed by alkaline peroxide workup afforded alcohol **13** in 89% yield as a single stereoisomer.[14] Oxidation of **13** followed by Wittig methylenation delivered olefin **14**. The stereochemistry of the newly generated stereogenic center was determined by NMR analysis of lactone **A** derived

(9) For reviews, see: (a) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457–2483. (b) Chemler, S. R.; Trauner, D.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 4544–4568. (c) Kotha, S.; Lahiri, K.; Kashinath, D. *Tetrahedron* **2002**, *58*, 9633–9695. (d) Suzuki, A. *Chem. Commun.* **2005**, 4759–4763.

(10) For recent reviews, see: (a) Hoveyda, A. H.; Zhugralin, A. R. *Nature* **2007**, *450*, 243–251. (b) Gradillas, A.; Perez-Castells, J. *Angew. Chem., Int. Ed.* **2006**, *45*, 6086–6101. (c) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4490–4527. (d) Deiters, A.; Martin, S. F. *Chem. Rev.* **2004**, *104*, 2199–2238.

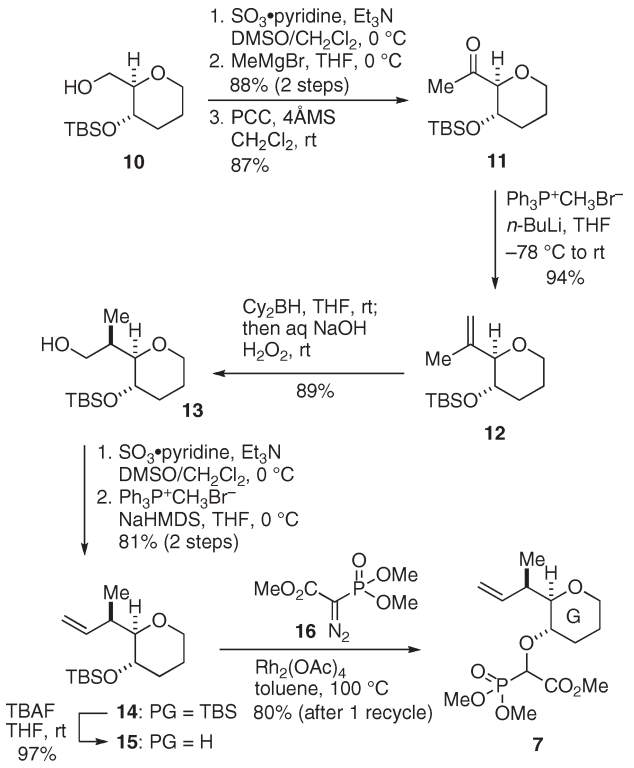
(11) Morita, M.; Haketa, T.; Koshino, H.; Nakata, T. *Org. Lett.* **2008**, *10*, 1679–1682.

(12) Nicolaou, K. C.; Hwang, C. K.; Marron, B. E.; DeFrees, S. A.; Couladouros, E. A.; Abe, Y.; Carroll, P. J.; Snyder, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 3040–3054.

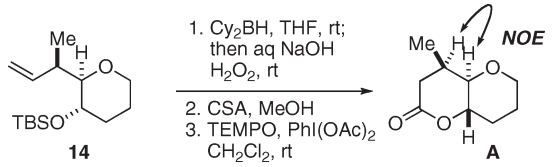
(13) Parikh, J. R.; Doering, W. v. E. *J. Am. Chem. Soc.* **1967**, *89*, 5505–5507.

(14) For a related example, see: Toshima, K.; Jyojima, T.; Yamaguchi, H.; Noguchi, Y.; Yoshida, T.; Murase, H.; Nakata, M.; Matsumura, S. *J. Org. Chem.* **1997**, *62*, 3271–3284.

SCHEME 3. Synthesis of Phosphonate 7



SCHEME 4. Derivatization of 14 to Lactone A



from olefin **14** (Scheme 4). The stereochemical outcome of the hydroboration could be explained by considering the conformation of olefin **12** (Figure 2). The TBS group within **14** was removed by treatment with TBAF, and the resultant alcohol **15** was reacted with diazophosphonate **16** in the presence of a catalytic amount of $Rh_2(OAc)_4$ in toluene at 100 °C to afford phosphonate **7** in good yield (80% yield after one recycle).[15]

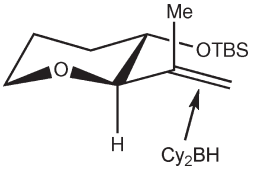
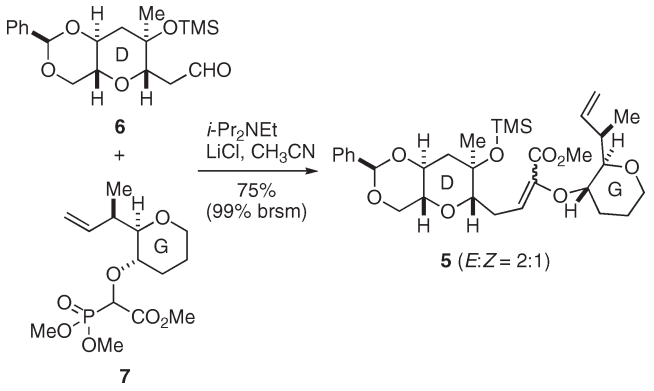


FIGURE 2. Plausible rationale for the stereochemical outcome of the hydroboration of olefin **12**.

Fragment Assembly and Investigations on the Stereoselective Construction of the C25 Stereogenic Center. We examined

SCHEME 5. HWE Coupling of 6 and 7



HWE coupling of aldehyde **6** and phosphonate **7** under several reaction conditions and found that the HWE coupling could be efficiently achieved under Masamune—Roush conditions (*i*-Pr_2NEt, LiCl, CH_3CN, room temperature)[16] to afford enoate **5** in 75% yield as a 2:1 mixture of geometric isomers, along with unreacted aldehyde **6** in 24% yield (99% yield based on recovered **6**) (Scheme 5). The *E*/*Z* isomers were partially (but not completely) separable by careful flash chromatography on silica gel. The major isomer was tentatively assigned as the *E* isomer, and this was confirmed at a later stage.

We next investigated stereoselective 1,4-reduction of enoate **5** as summarized in Table 1. Enoate **5** was used as a 1:1 mixture of *E*/*Z* isomers. Treatment of **5** with magnesium metal in MeOH gave a 1:3 mixture of **4** and 25-*epi*-**4** in 73% combined yield (entry 1). 1,4-Reduction of **5** by the action of $NiCl_2/NaBH_4$ in wet MeOH[17] accompanied concomitant reduction of the terminal olefin, giving a 1:2 mixture of **16** and 25-*epi*-**16** in 82% yield (entry 2). An attempt to reduce **5** with L-Selectride (EtOH, THF, −70 °C) did not yield any 1,4-reduction product (entry 3). Exposure of **5** to SmI_2 in THF/MeOH (4:1) at 0 °C gave a 1:5 mixture of **4** and 25-*epi*-**4** in 91% yield (entry 4). We also found that the *E*/*Z* ratio of **5** did not affect the stereochemical outcome of its 1,4-reduction with SmI_2/MeOH (entry 5). Reduction of **5** with SmI_2/MeOH in the presence of HMPA (THF, −78 °C) yielded a 1:2 mixture of **4** and 25-*epi*-**4** in 97% yield (entry 6). Esters **4** and 25-*epi*-**4** were separable by flash chromatography on silica gel.

The stereochemistry of the major diastereomer 25-*epi*-**4** was confirmed by its derivatization to lactone 25-*epi*-**3** in a three-step sequence involving deprotection of the TMS group, saponification of the methyl ester, and ensuing lactonization under Yamaguchi conditions[18] (Scheme 6). The observed NOE enhancement between 25-H and the C21 methyl group supported the undesired stereochemistry of the C25 stereogenic center. Unfortunately, attempts to epimerize ester 25-*epi*-**4** or lactone 25-*epi*-**3** by base treatment were not feasible.

These disappointing results could be ascribed to the intrinsic substrate bias of enoate **5**; the *si* faces of (*E*)-**5** and

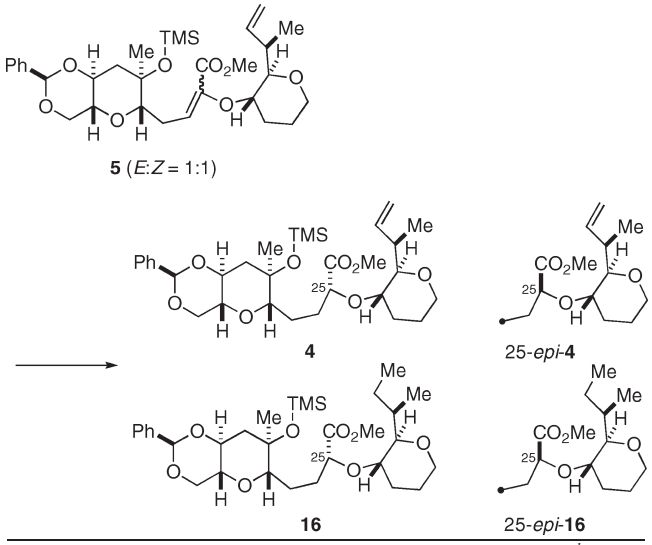
(15) (a) Paquet, F.; Sinay, P. *Tetrahedron Lett.* **1984**, *25*, 3071–3074. (b) Moyer, M. P.; Feldman, P. L.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 5223–5330. (c) Moody, C. J.; Sie, E.-R. H. B.; Kulagowski, J. J. *Tetrahedron* **1992**, *48*, 3991–4004. (d) Cox, G. G.; Miller, D. J.; Moody, C. J.; Sie, E.-R. H. B.; Kulagowski, J. J. *Tetrahedron* **1994**, *50*, 3195–3212.

(16) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183–2186.

(17) Khurana, J. M.; Sharma, P. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 549–552.

(18) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993.

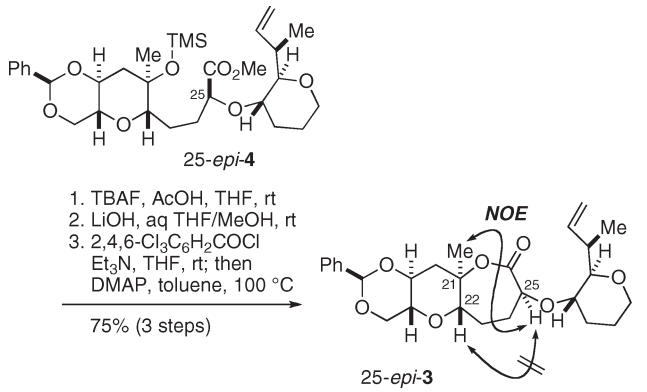
TABLE 1. Screening of Reaction Conditions for 1,4-Reduction of 5



entry[a]	reagents and conditions	products (yield,[b] dr[c])
1	Mg, MeOH, room temperature	**4** + 25-*epi*-**4** (73%, 1:3)
2	$NiCl_2$, $NaBH_4$, MeOH, 0 °C	**16** + 25-*epi*-**16** (82%, 1:2)
3	L-Selectride, EtOH, THF, −70 °C	no reaction
4	SmI_2, THF/MeOH (4:1), 0 °C	**4** + 25-*epi*-**4** (91%, 1:5)
5[d]	SmI_2, THF/MeOH (4:1), 0 °C	**4** + 25-*epi*-**4** (90%, 1:5)
6	SmI_2, HMPA, THF/MeOH (4:1), −78 °C	**4** + 25-*epi*-**4** (97%, 1:2)

[a]Unless otherwise stated, all reactions were carried out using **5** (*E*:*Z* = 1:1). [b]Yields are based on a purified mixture of **4** and 25-*epi*-**4** (or **16** and 25-*epi*-**16**). [c]Diastereomer ratios were determined on the basis of ^{1}H NMR analysis (500 MHz). [d]The reaction was carried out using **5** (*E*:*Z* = 2:5).

SCHEME 6. Derivatization of 25-*epi*-4 to 25-*epi*-3



(*Z*)-**5** would be sterically encumbered by the G-ring tetrahydropyran (Figure 3).

We have also explored 1,4-reduction of α,β-unsaturated lactone **17** to see whether the cyclic constraint of the seven-membered lactone ring might be exploited to construct the C25 stereogenic center in a stereoselective fashion (Scheme 7). Deprotection of the TMS group within enoate **5** (*E*:*Z* = 3:1) gave alcohols (*E*)-**18** and (*Z*)-**18**, which were separable by flash chromatography on silica gel. These alcohols were individually hydrolyzed to give the respective carboxylic acids (*E*)-**19** and (*Z*)-**19**. Lactonization of acid (*E*)-**19** under Yamaguchi conditions afforded α,β-unsaturated lactone **17** in 98% yield. By contrast, the desired lactone **17** was

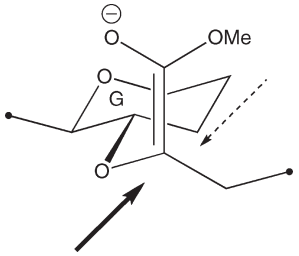


FIGURE 3. Plausible rationale for the stereochemical outcome of the 1,4-reduction of enoate **5**. The geometry of the double bond is arbitrary.

obtained in only 20% yield from acid (*Z*)-**19**. We reasoned the formation of lactone **17** from acid (*Z*)-**19** occurred via partial generation of a ketene from the intermediary activated mixed anhydride.[19] From the reactivity difference between acids (*E*)-**19** and (*Z*)-**19**, we concluded that the major isomer obtained in the HWE coupling of aldehyde **6** and phosphonate **7** was (*E*)-**5**. Unfortunately, our efforts to reduce α,β-unsaturated lactone **17** in a chemo- and stereoselective manner gave only disappointing results. For example, 1,4-reduction of α,β-unsaturated lactone **17** with SmI_2 in THF/MeOH at 0 °C gave only a complex mixture of unidentified products, whereas hydrogenation of **17** (H_2 (0.8 MPa), THF/MeOH, room temperature) proceeded with poor diastereoselectivity (dr 1.4:1) and accompanied saturation of the terminal olefin.

The difficulties encountered during the investigations on 1,4-reduction of ester **5** and α,β-unsaturated lactone **17** led us to revise the initial synthetic plan toward the DEFG-ring skeleton **1**.

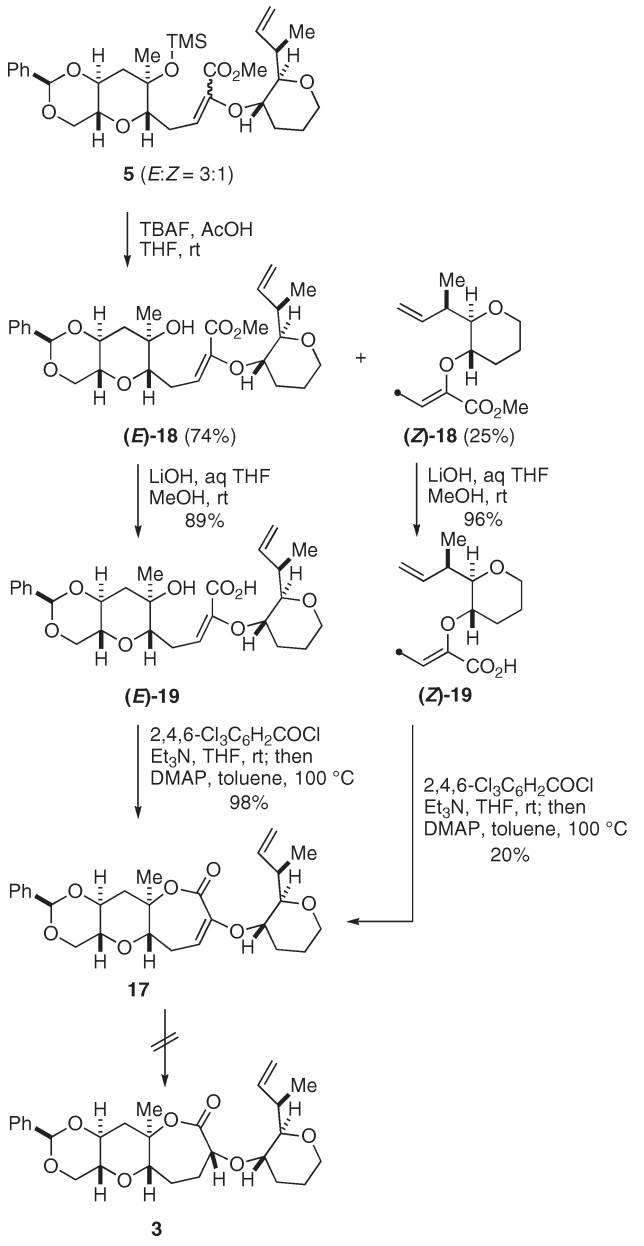
Second-Generation Synthesis Plan toward the DEFG-Ring Skeleton. At this stage, it occurred to us that the C25 stereogenic center could be defined by making use of the intrinsic substrate bias of endocyclic enol ethers. More specifically, we envisioned stereoselective hydroboration of seven-membered endocyclic enol ether **20** as a suitable means to settle the C25 stereogenic center, and the resultant alcohol **21** would be easily elaborated to ester **4**, the key intermediate in the first-generation synthesis plan, via oxidative cleavage of the seven-membered ether ring (Scheme 8). Endocyclic enol ether **20** was planned to be synthesized from the D-ring olefin **22** and the (F)G-ring enol phosphate **23** via Suzuki—Miyaura coupling.[20]

Synthesis of the D-Ring Olefin 22. The D-ring olefin **22** was synthesized from silyl ether **9** (Scheme 9). A two-stage reduction of **9** gave alcohol **24** in a nearly quantitative yield.

(19) For an example, see: Fürstner, A.; Nevado, C.; Waser, M.; Tremblay, M.; Chevrier, C.; Teplý, F.; Aïssa, C.; Moulin, E.; Müller, O. *J. Am. Chem. Soc.* **2007**, *129*, 9150–9161.

(20) For the synthesis of polycyclic ethers based on Suzuki—Miyaura coupling, see: (a) Sasaki, M.; Fuwa, H.; Inoue, M.; Tachibana, K. *Tetrahedron Lett.* **1998**, *39*, 9027–9031. (b) Sasaki, M.; Fuwa, H.; Ishikawa, M.; Tachibana, K. *Org. Lett.* **1999**, *1*, 1075–1077. (c) Sasaki, M.; Ishikawa, M.; Fuwa, H.; Tachibana, K. *Tetrahedron* **2002**, *58*, 1889–1911. (d) Fuwa, H.; Kainuma, N.; Tachibana, K.; Sasaki, M. *J. Am. Chem. Soc.* **2002**, *124*, 14983–14992. (e) Tsukano, C.; Ebine, M.; Sasaki, M. *J. Am. Chem. Soc.* **2005**, *127*, 4326–4335. (f) Fuwa, H.; Ebine, M.; Bourdelais, A. J.; Baden, D. G.; Sasaki, M. *J. Am. Chem. Soc.* **2006**, *128*, 16989–16999. (g) Nicolaou, K. C.; Frederick, M. O.; Burtoloso, A. C. B.; Denton, R. M.; Rivas, F.; Cole, K. P.; Aversa, R. J.; Gibe, R.; Umezawa, T.; Suzuki, T. *J. Am. Chem. Soc.* **2008**, *130*, 7466–7476. (h) Nicolaou, K. C.; Aversa, R. J.; Jin, J.; Rivas, F. *J. Am. Chem. Soc.* **2010**, *132*, 6855–6861. See also: (i) Sasaki, M.; Fuwa, H. *Nat. Prod. Rep.* **2008**, *25*, 401–426. (j) Sasaki, M.; Fuwa, H. *Synlett* **2004**, 1851–1874.

SCHEME 7. Synthesis of α,β-Unsaturated Lactone 17 and Its Attempted Reduction

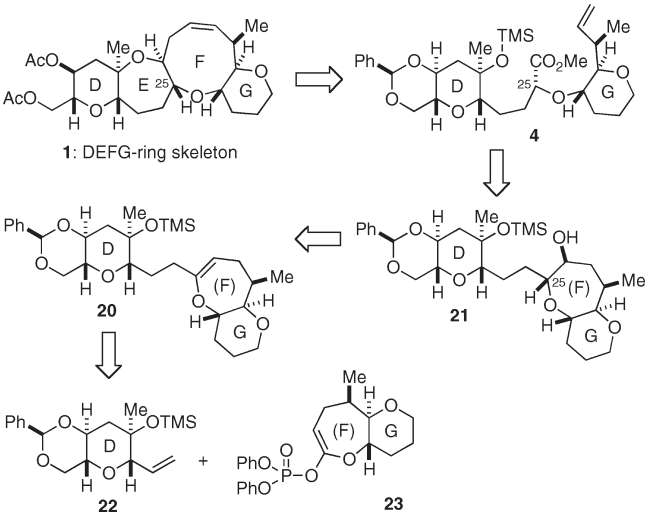


Mesylation of **24** and treatment with $PhSeSePh/NaBH_4$ yielded selenide **25** in 96% yield for the two steps. Finally, oxidation of **25** with *m*CPBA and in situ treatment with Et_3N afforded olefin **22** in 85% yield (90% based on recovered **25**).

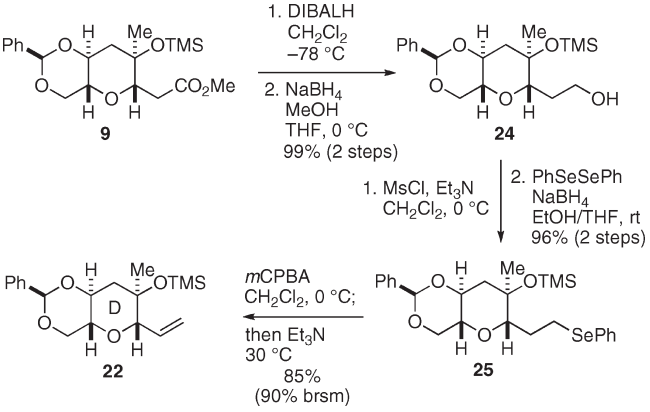
Synthesis of the (F)G-Ring Enol Phosphate 23. The synthesis of the (F)G-ring enol phosphate **23** commenced with oxidation of alcohol **13** followed by Wittig reaction using $Ph_3P{=}CHCO_2Et$ to give enoate **26** in 87% yield for the two steps (Scheme 10). Hydrogenation of **26** gave ester **27** in 100% yield. Reduction of **27** with $LiAlH_4$ delivered alcohol **28** (100% yield), which was treated with acidic methanol to remove the TBS group to afford diol **29** in 100% yield. Oxidative lactonization of **29** with $TEMPO/PhI(OAc)_2$[21] led

(21) Ebine, M.; Suga, Y.; Fuwa, H.; Sasaki, M. *Org. Biomol. Chem.* **2010**, *8*, 39–42.

SCHEME 8. Second-Generation Approach toward the DEFG-Ring Skeleton 1



SCHEME 9. Synthesis of Olefin 22



to seven-membered lactone **30** in 90% yield, which was treated with KHMDS in the presence of $(PhO)_2P(O)Cl$[22] to furnish the (F)G-ring enol phosphate **23**.

Fragment Assembly and Stereoselective Synthesis of Ester 4. The fragments **22** and **23** were assembled by means of Suzuki—Miyaura coupling (Scheme 11). Thus, hydroboration of olefin **22** with 9-BBN-H in THF at room temperature generated an alkylborane. This was, without isolation, treated with aqueous Cs_2CO_3 and then with enol phosphate **23** and $Pd(PPh_3)_4$ (10 mol %) in DMF at 50 °C, giving rise to endocyclic enol ether **20** in 96% yield. Hydroboration of **20** with $BH_3 \cdot SMe_2$ followed by alkaline oxidative workup gave a 2:1 mixture of diastereomeric alcohols **21** and **21′** in 100% yield. Although we found that epoxidation of **20** with dimethyldioxirane (DMDO) and in situ reduction of the resultant epoxide[23] proceeded in a stereoselective manner, the product yield was moderate (30−54% yield), and several unidentified byproducts

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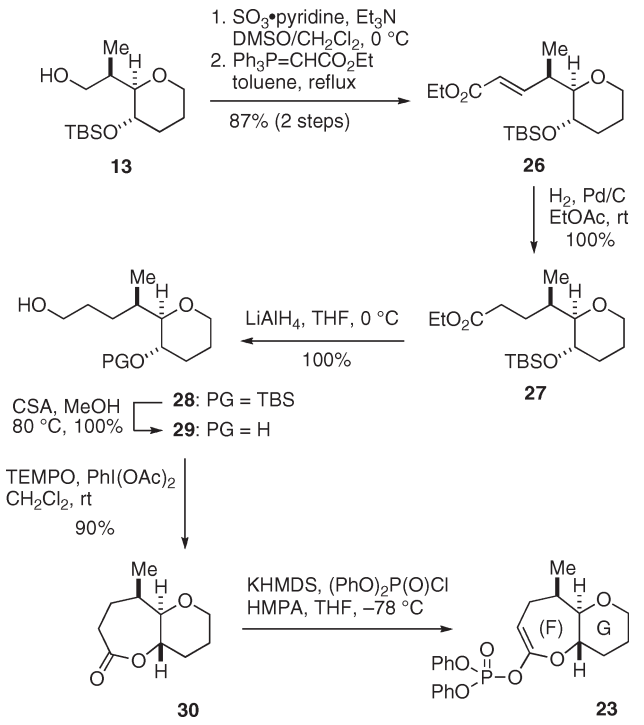
(23) (a) Fujiwara, K.; Awakura, D.; Tsunashima, M.; Nakamura, A.; Honma, T.; Murai, A. *J. Org. Chem.* **1999**, *64*, 2616–2617. (b) Allwein, S. P.; Cox, J. M.; Howard, B. E.; Johnson, H. W. B.; Rainier, J. D. *Tetrahedron* **2002**, *58*, 1997–2009. (c) Orendt, A. M.; Roberts, S. W.; Rainier, J. D. *J. Org. Chem.* **2006**, *71*, 5565–5573.

SCHEME 10. Synthesis of Enol Phosphate 23

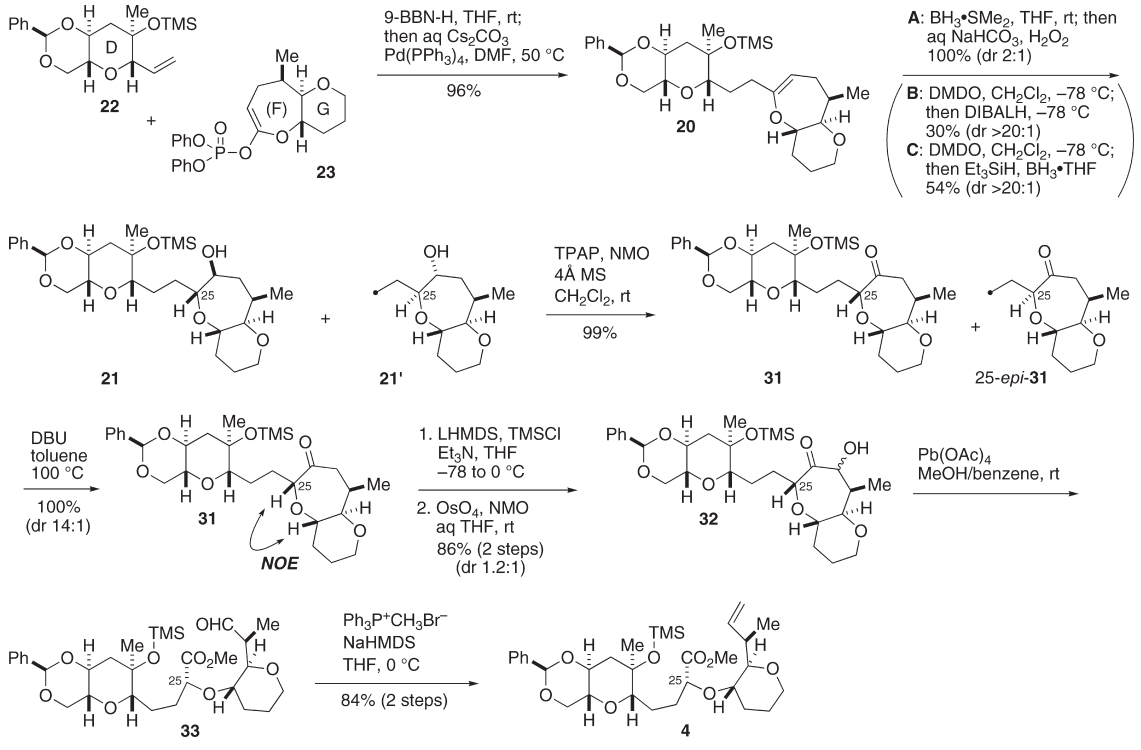


were generated alongside. Therefore, we examined base-promoted epimerization after oxidization of the diastereomeric mixture of alcohols **21** and **21′** with TPAP/NMO[24] in 99% yield. Gratifyingly, treatment of a 2:1 mixture of **31** and 25-*epi*-**31** with excess DBU in toluene at 100 °C resulted in significant enrichment of the desired **31** (dr 14:1) with quantitative material recovery. The minor diastereomer at C25 was removed at a later stage. The stereochemistry of the C25 stereogenic center was established by an NOE experiment as shown.

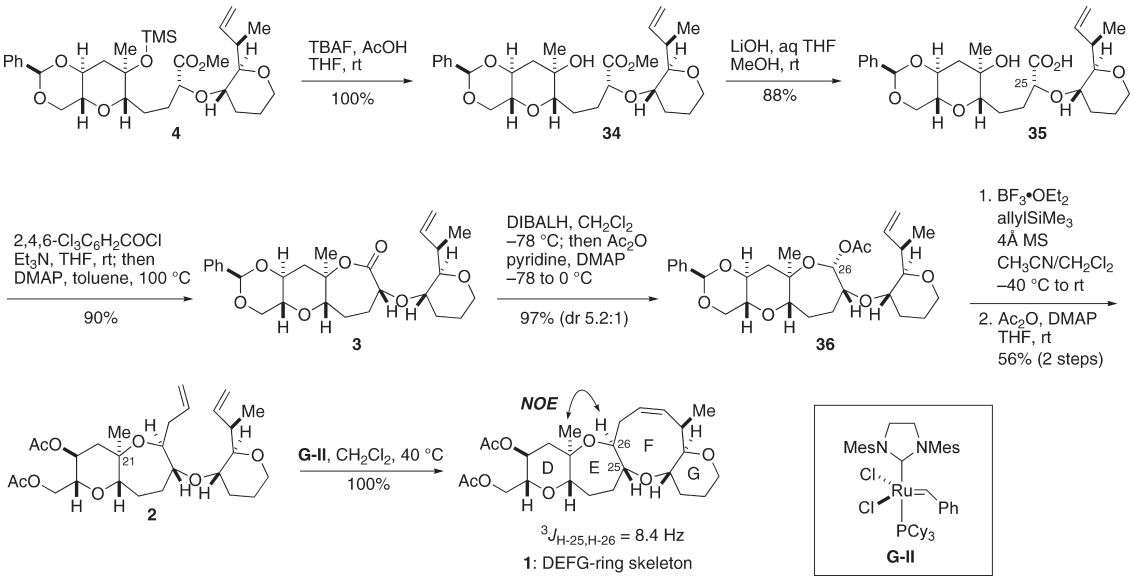
Having defined the C25 stereogenic center, ketone **31** was elaborated to the key intermediate ester **4** via oxidative cleavage of the seven-membered ether ring. Enolization of ketone **31** with LHMDS in the presence of TMSCl/Et_3N gave the corresponding enol silane, which was oxidized with OsO_4/NMO to deliver α-hydroxy ketone **32** as an inconsequential 1.2:1 mixture of diastereomers (86% yield for the two steps). Treatment of **32** with $Pb(OAc)_4$ in the presence of $NaHCO_3$ in MeOH/benzene resulted in a cleavage of the seven-membered ether ring. The resultant aldehyde-ester **33** was immediately exposed to $Ph_3P{=}CH_2$ to afford ester **4** in 84% yield for the two steps. The stereochemical integrity of the C25 stereogenic center was unaffected during these synthetic manipulations, as judged by ^1H NMR analysis of ester **4**. Thus, we were able to synthesize the key intermediate ester **4** in a highly stereocontrolled manner.

Completion of the Synthesis of the DEFG-Ring Skeleton 1. Elaboration of ester **4** into the targeted DEFG-ring skeleton **1** is summarized in Scheme 12. Desilylation of **4** with TBAF/AcOH gave alcohol **34** in 100% yield. Saponification of the methyl ester of **34** yielded hydroxy acid **35** in 88% yield. At this stage, the minor C25 epimer was removed by flash chromatography on silica gel. Lactonization of **35** under Yamaguchi conditions afforded seven-membered lactone **3** in 90% yield. Reduction of **3** with DIBALH and in situ acetylation according to the Rychnovsky protocol[25] gave α-acetoxy ether **36** in 97% yield as a 5.2:1 mixture of diastereomers. The stereochemistry of the C26 stereogenic center of the major isomer was tentatively assigned as *R* on the basis of our previous experience.[7e,g,h] We then examined stereoselective allylation of **36** with allyltrimethylsilane in the presence of Lewis acid under various conditions. The best

SCHEME 11. Stereoselective Synthesis of Ester 4



SCHEME 12. Completion of the Synthesis of the DEFG-Ring Skeleton 1



result was obtained when **36** was treated with $BF_3 \cdot OEt_2$ and excess allyltrimethylsilane in the presence of 4 Å molecular sieves in CH_3CN/CH_2Cl_2 at −40 °C to room temperature. This allylation was accompanied by cleavage of the benzylidene acetal to give a diol. After acetylation of the resultant diol, diene **2** was isolated in 56% yield for the two steps as a single stereoisomer. The stereochemical outcome of the allylation can be ascribed to the presence of the axially disposed C21 methyl group, which would preclude axial attack of allyltrimethylsilane and enforce the allylation to proceed from the sterically less encumbered β-face of the molecule. Finally, RCM of diene **2** by its exposure to the Grubbs second-generation catalyst (**G-II**)[26] in CH_2Cl_2 at 40 °C furnished the tetracyclic ether **1** in 100% yield, which represents the DEFG-ring skeleton of GAs. The stereochemistry of **1** was confirmed by an NOE experiment and $^3J_{H,H}$ analysis as shown.

Conformational Analysis of the Tetracyclic Ether 1. The ^{1}H NMR spectrum of the tetracyclic ether **1** recorded in $CDCl_3$, C_6D_6, or CD_3OD/C_5D_5N (1:1) at 293 K showed a single set of sharp signals, which is in accordance with that of natural GAA measured in CD_3OD/C_5D_5N (1:1) at 293 K.[1b] This observation suggests that **1** exists as a single stable conformer. However, this is in sharp contrast to our previous finding on the ^{1}H NMR spectrum (C_5D_5N, 298 K) of a structurally related tetracyclic ether **37** representing the E′FGH-ring skeleton of ciguatoxins (Figure 4),[27] wherein the signals of the olefinic and the neighboring methylene protons significantly broadened due to the slow conformational change of the FG-ring moiety.[28] More specifically, tetracyclic ether **37** exists as an interconvertable mixture of UP and DOWN conformers, both of which had been successfully characterized by recording NMR spectra at low temperature. Such conformational behavior had also been reported for the F-ring of ciguatoxins.[29] Furthermore, recent studies by Hirama, Inoue, and co-workers indicated that the conformational flexibility of the F-ring of ciguatoxins is critically important for their potent biological activities.[30] Intrigued by the striking conformational difference observed between **1** and **37**, we analyzed the conformation of **1** on the basis of $^3J_{H,H}$ values and NOESY correlations (Figure 5) and found that **1** adopts UP conformation exclusively. Molecular modeling studies[31] suggested that the DOWN conformer would suffer from severe transannular interactions between the C30 methyl group and H-27 and H-32, while such unfavorable nonbonding interactions would not exist in the UP conformer (Figure 4).

Thus, the NMR spectroscopic analysis and molecular modeling indicated that the F-ring of **1** exclusively adopts the UP conformer, where the C30 methyl group acts as a conformational locking device and does not undergo slow conformational change as observed for ciguatoxins and the E′FGH-ring model compound **37**. This result suggests that GAA would also exist solely as an UP conformer.

Conclusion

Although our initially designed strategy based on the Horner—Wadsworth—Emmons coupling/1,4-reduction

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(25) Dahanukar, V. H.; Rychnovsky, S. D. *J. Org. Chem.* **1996**, *61*, 8317–8320.
(26) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953–956.
(27) (a) Sasaki, M.; Noguchi, T.; Tachibana, K. *Tetrahedron Lett.* **1999**, *40*, 1337–1340. (b) Sasaki, M.; Noguchi, T.; Tachibana, K. *J. Org. Chem.* **2002**, *67*, 3301–3310.
(28) For other examples, see: (a) Inoue, M.; Sasaki, M.; Tachibana, K. *Tetrahedron Lett.* **1997**, *38*, 1611–1614. (b) Inoue, M.; Sasaki, M.; Tachibana, K. *Tetrahedron* **1999**, *55*, 10949–10970. (c) Imai, H.; Uehara, H.; Inoue, M.; Oguri, H.; Oishi, T.; Hirama, M. *Tetrahedron Lett.* **2001**, *42*, 6219–6222. (d) Inoue, M.; Wang, G. X.; Wang, J.; Hirama, M. *Org. Lett.* **2002**, *4*, 1183–1186. (e) Takai, S.; Isobe, M. *Org. Lett.* **2002**, *4*, 1183–1186. (f) Takai, S.; Sawada, N.; Isobe, M. *J. Org. Chem.* **2003**, *68*, 3225–3231.
(29) Murata, M.; Legrand, A.-M.; Ishibashi, Y.; Fukui, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1990**, *112*, 4380–4386.
(30) (a) Inoue, M.; Lee, N.; Miyazaki, K.; Usuki, T.; Matsuoka, S.; Hirama, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 8611–8614. (b) Ishihara, Y.; Lee, N.; Oshiro, N.; Matsuoka, S.; Yamashita, S.; Inoue, M.; Hirama, M. *Chem. Commun.* **2010**, *46*, 2968–2970.
(31) The UP and DOWN conformers of the DEFG-ring model compound **1** were generated by conformational searches using MMFF force field (*CONFLEX ver. 6.7*; CONFLEX Corporation: Tokyo, Japan) and geometrically optimized at the HF/6-31G*//PM3 level of theory (*Spartan '08*; Wavefunction, Inc.: Irvine, CA).

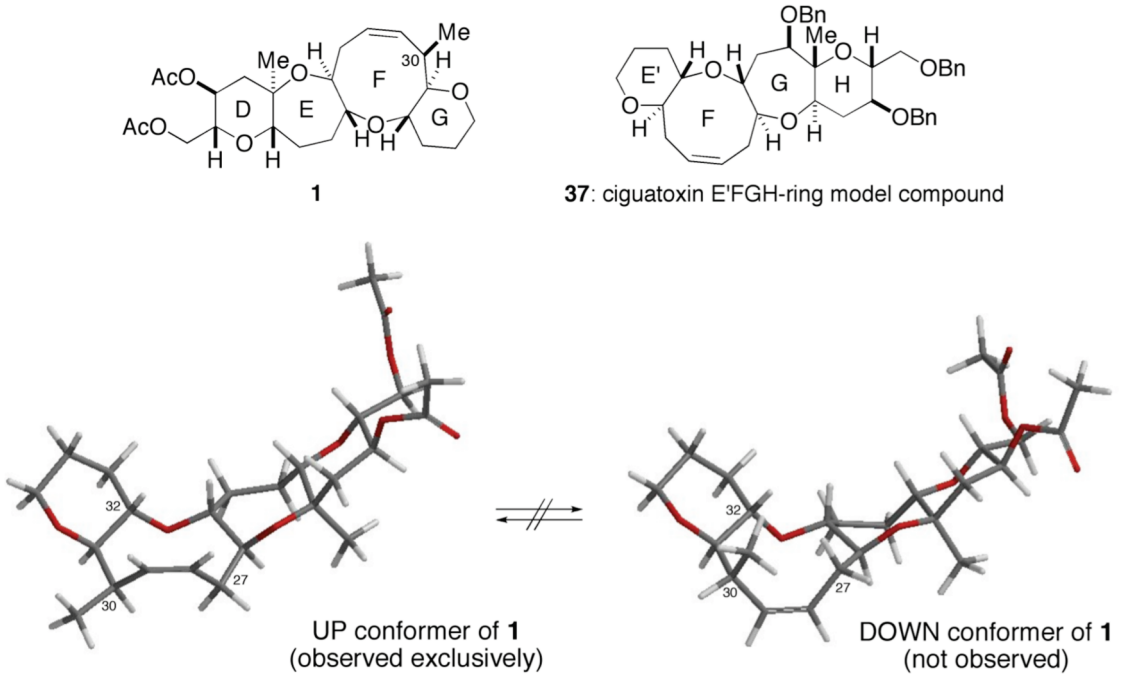

FIGURE 4. Conformational isomers of **1** and structure of **37**.

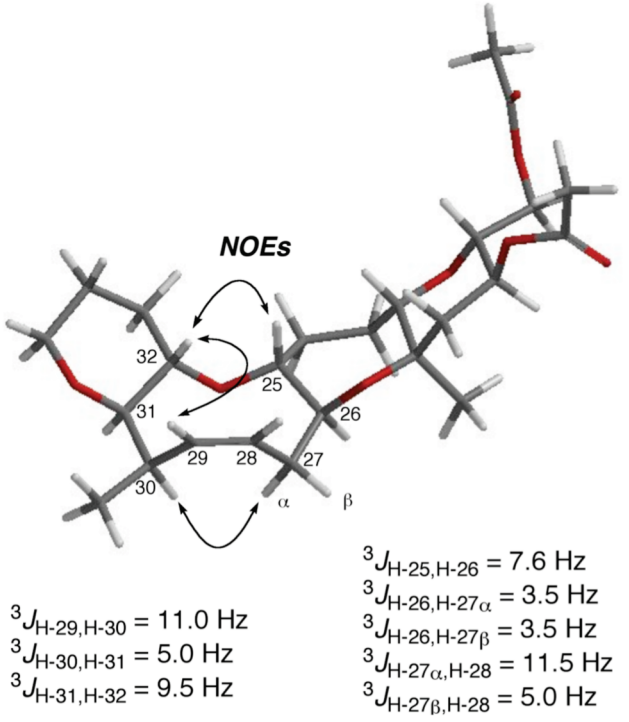

FIGURE 5. Conformational analysis of **1** (600 MHz ^{1}H NMR, C_6D_6).

sequence turned out to be unrewarding, we have eventually succeeded in the synthesis of the DEFG-ring skeleton **1** of gambieric acids in a highly stereocontrolled manner. Suzuki—Miyaura coupling was utilized to assemble the D-ring olefin **22** and the (F)G-ring enol phosphate **23**, which afforded endocyclic enol ether **20**. The C25 stereogenic center was successfully defined during the three-step transformation (hydroboration/oxidation/epimerization) from endocyclic enol ether **20** to ketone **31**. The seven-membered ether ring was then oxidatively cleaved to yield the key intermediate ester **4**. The E-ring was constructed via Yamaguchi lactonization and stereoselective allylation as the key steps. Finally, the F-ring was forged by RCM to complete the synthesis of the DEFG-ring skeleton **1**. Despite its structural similarity to our previously synthesized ciguatoxin E′FGH-ring model compound **37**, the tetracyclic ether **1** was found to exist as a single stable conformer in solution, where the C30 methyl group acts as a conformational locking device. Further studies toward the total synthesis of gambieric acids are currently underway in our laboratory.

Experimental Section

Endocyclic Enol Ether 20. To a solution of lactone **30** (62.5 mg, 0.340 mmol) in THF (3 mL) were added HMPA (0.18 mL, 1.0 mmol) and $(PhO)_2P(O)Cl$ (0.090 mL, 0.41 mmol), and the solution was cooled to −78 °C. To this solution was added KHMDS (0.5 M solution in toluene, 0.90 mL, 0.45 mmol), and the resultant solution was stirred at −78 °C for 1.5 h. The reaction was quenched with 3% aqueous ammonia solution. The resultant mixture was diluted with diethyl ether and allowed to warm to room temperature over a period of 20 min. The resultant mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was passed through a pad of silica gel (eluted with 20% EtOAc/hexanes) to give enol phosphate **23**, which was immediately used in the next reaction.

To a solution of olefin **22** (76.4 mg, 0.220 mmol) in THF (2 mL) was added a freshly prepared solution of 9-BBN-H dimer (75 mg, 0.31 mmol) in THF (1 mL), and the resultant solution was stirred at room temperature for 3 h. To this solution was added 3 M aqueous Cs_2CO_3 solution (0.22 mL, 0.66 mmol), and the resultant mixture was stirred at room temperature for 20 min. To this mixture were added a solution of the above

crude enol phosphate **23** in DMF (1 mL + 0.5 mL rinse) and $Pd(PPh_3)_4$ (25.4 mg, 0.0220 mmol). The resultant mixture was stirred at 50 °C for 17 h. After being cooled to room temperature, the reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (3% to 5% EtOAc/hexanes) gave endocyclic enol ether **20** (109.2 mg, 96%) as a colorless oil: $[\alpha]^{20}_D$ −41.2 (*c* 0.37, C_6H_6); IR (film) 2939, 1455, 1250, 1097, 1016, 867, 839, 751, 417 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 7.67 (d, J = 7.5 Hz, 2H), 7.20 (dd, J = 7.5, 7.5 Hz, 2H), 7.12 (m, 1H), 5.35 (s, 1H), 4.70 (dd, J = 6.0, 6.0 Hz, 1H), 4.26 (dd, J = 10.0, 4.0 Hz, 1H), 3.75—3.65 (m, 2H), 3.53 (dd, J = 10.0, 10.0 Hz, 1H), 3.39—3.27 (m, 3H), 3.23 (dd, J = 9.0, 4.5 Hz, 1H), 3.02 (ddd, J = 12.0, 12.0, 2.0 Hz, 1H), 2.44 (ddd, J = 15.0, 10.0, 5.0 Hz, 1H), 2.32 (m, 1H), 2.27—2.18 (m, 2H), 2.18—2.08 (m, 2H), 2.05 (br d, J = 10.0 Hz, 1H), 2.01 (d, J = 6.0 Hz, 1H), 1.95 (m, 1H), 1.63 (dddd, J = 15.0, 10.0, 10.0, 5.0 Hz,1H), 1.53 (dddd, J = 12.0, 12.0, 12.0, 4.0 Hz, 1H), 1.42 (ddddd, J = 13.0, 13.0, 13.0, 4.5, 4.5 Hz, 1H), 1.23 (m, 1H), 1.21 (s, 3H), 1.09 (d, J = 7.0 Hz, 3H), 0.13 (s, 9H); ^{13}C NMR (125 MHz, C_6D_6) δ 159.5, 138.7, 128.9, 128.5, 128.3, 126.8 (2C), 104.5, 101.8, 85.4, 84.9, 76.9, 76.2, 74.9, 74.3, 69.6, 67.7, 46.0, 35.2, 33.3, 31.5, 29.1, 26.7, 26.4, 22.7, 14.4, 2.6 (3C); HRMS (ESI) calcd for $C_{29}H_{45}O_6Si$ $[(M + H)^+]$ 517.2980, found 517.2987.

Alcohols 21 and 21′. To a solution of endocyclic enol ether **20** (10.6 mg, 0.0205 mmol) in THF (1 mL) cooled to 0 °C was added $BH_3 \cdot SMe_2$ (1.9 M solution in THF, 0.11 mL, 0.21 mmol), and the resultant solution was stirred at room temperature for 70 min. To this solution cooled to 0 °C were added saturated aqueous $NaHCO_3$ solution (0.5 mL) and 30% aqueous H_2O_2 solution (0.3 mL), and the resultant mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layer was washed with saturated aqueous $Na_2S_2O_3$ solution and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (20 to 25% EtOAc/hexanes) gave an approximately 2:1 mixture of alcohol **21** and its diastereomer **21′** (11.2 mg, 100%) as a colorless oil: $[\alpha]^{16}_D$ −10.1 (*c* 1.43, $CHCl_3$); IR (film) 3457, 2937, 1454, 1376, 1251, 1141, 1045, 867, 752, 678 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, signals for the major diastereomer) δ 7.46 (dd, J = 8.0, 1.5 Hz, 2H), 7.37—7.29 (m, 3H), 5.49 (s, 1H), 4.28 (dd, J = 10.5, 5.0 Hz, 1H), 3.89 (m, 1H), 3.65 (dd, J = 10.5, 10.5 Hz, 1H), 3.60 (m, 1H), 3.50 (ddd, J = 13.0, 9.5, 4.5 Hz, 1H), 3.34 (ddd, J = 10.0, 10.0, 5.0 Hz, 1H), 3.10—3.08 (m, 4H), 3.03 (dd, J = 9.5, 5.5 Hz, 1H), 2.26—2.14 (m, 2H), 2.07 (m, 1H), 2.03—1.80 (m, 5H), 1.70—1.60 (m, 2H), 1.52—1.39 (m, 2H), 1.39—1.22 (m, 2H), 1.27 (s, 3H), 1.09 (d, J = 7.0 Hz, 3H), 0.10 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$, signals for the major diastereomer) δ 129.1, 128.33, 128.31 (2C), 126.2 (2C), 101.8, 87.3, 86.0, 84.8, 79.1, 76.8, 76.5, 74.2, 73.9, 69.5, 68.2, 45.4, 38.2, 33.4, 32.4, 31.3, 26.2, 25.3, 22.4, 15.0, 2.6 (3C); HRMS (ESI) calcd for $C_{29}H_{46}O_7SiNa$ $[(M + Na)^+]$ 557.2911, found 557.2915.

Ketone 31. To a solution of an approximately 2:1 mixture of alcohol **21** and its diastereomer **21′** (633.6 mg, 1.19 mmol) in CH_2Cl_2 (11 mL) were added 4 Å molecular sieves (ca. 0.6 g), NMO (697 mg, 5.95 mmol), and TPAP (42 mg, 0.12 mmol), and the resultant mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc and passed through a pad of silica gel. The filtrate was concentrated under reduced pressure to give an approximately 2:1 mixture of ketone **31** and its C25-epimer 25-*epi*-**31** (620.9 mg, 99%).

To a solution of an approximately 2:1 mixture of ketone **31** and its C25-epimer 25-*epi*-**31** (0.60 g, 1.1 mmol) in toluene (11 mL) was added DBU (1.67 mL, 11.2 mmol), and the resultant solution was heated at 100 °C for 14.5 h. After being cooled to room temperature, the reaction mixture was directly purified by flash chromatography on silica gel (10 to 15% EtOAc/hexanes) to give diastereomerically enriched ketone **31** (0.61 g, 100%, dr 14:1 based on 500 MHz ^1H NMR analysis) as a colorless oil: $[\alpha]^{16}_D$ +43.1 (*c* 0.88, $CHCl_3$); IR (film) 2955, 1711, 1454, 1377, 1250, 1098, 1046, 867, 840, 753, 698 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 7.67 (d, J = 7.5 Hz, 2H), 7.21 (dd, J = 7.5, 7.5 Hz, 2H), 7.12 (m, 1H), 5.34 (s, 1H), 4.19 (dd, J = 10.0, 5.0 Hz, 1H), 3.64 (dd, J = 11.5, 4.5 Hz, 1H), 3.55 (dd, J = 9.0, 4.0 Hz, 1H), 3.50 (dd, J = 10.0, 10.0 Hz, 1H), 3.33 (ddd, J = 12.5, 10.0, 4.5 Hz, 1H), 3.26 (ddd, J = 10.0, 10.0, 5.0 Hz, 1H), 3.21 (dd, J = 10.0, 2.0 Hz, 1H), 3.09 (dd, J = 9.5, 5.0 Hz, 1H), 3.00 (ddd, J = 11.5, 11.5, 2.5 Hz, 1H), 2.90 (ddd, J = 9.5, 9.5, 5.0 Hz, 1H), 2.85 (dd, J = 12.0, 2.0 Hz, 1H), 2.22 (dd, J = 11.0, 4.5 Hz, 1H), 2.20—2.13 (m, 2H), 2.13—2.00 (m, 2H), 1.92 (dd, J = 11.0, 11.0 Hz, 1H), 1.87 (m, 1H), 1.64 (m, 1H), 1.53 (m, 1H), 1.40—1.25 (m, 2H), 1.25 (s, 3H), 1.15 (m, 1H), 0.98 (d, J = 7.5 Hz, 3H), 0.13 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 216.4, 137.5, 129.1, 128.3 (2C), 126.2 (2C), 101.7, 87.6, 85.6, 83.7, 76.7, 75.3, 74.2, 73.8, 69.5, 67.6, 45.4, 44.1, 32.1, 31.1, 30.7, 25.6, 24.7, 22.4, 12.3, 2.5 (3C); HRMS (ESI) calcd for $C_{29}H_{45}O_7Si$ $[(M + H)^+]$ 533.2929, found 533.2938.

α-Hydroxy Ketone 32. To a solution of ketone **31** (453.5 mg, 0.8524 mmol, ca. 14:1 mixture of diastereomers at C25 stereogenic center) in THF (8 mL) were added TMSCl (2.20 mL, 17.3 mmol) and Et_3N (2.30 mL, 16.5 mmol), and the resultant solution was cooled to −78 °C. To this solution was added freshly prepared LHMDS (1.0 M solution in THF, 4.30 mL, 4.30 mmol), and the resultant solution was stirred at −78 °C for 10 min and then allowed to warm to 0 °C over a period of 45 min. The reaction was quenched with aqueous pH 7 phosphate buffer. The resultant mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude enol silane was immediately used in the next reaction.

To a solution of the above crude material in THF/H_2O (4:1, v/v, 8 mL) were added NMO (0.35 mL, 1.7 mmol) and OsO_4 (0.039 M solution in *t*-BuOH, 2.1 mL, 0.082 mmol), and the resultant mixture was stirred at room temperature for 14 h. The reaction was quenched with saturated aqueous Na_2SO_3 solution. The resultant mixture was extracted with EtOAc, and the organic layer was washed with H_2O and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (20 to 25% EtOAc/hexanes) gave α-hydroxy ketone **32** (401.7 mg, 86% for two steps) as a 1.2:1 mixture of diastereomers. These diastereomers were separable upon careful flash chromatography on silica gel. Data for major diastereomer: $[\alpha]^{26}_D$ +48.9 (*c* 1.53, $CHCl_3$); IR (film) 3480, 2941, 2857, 1712, 1377, 1251, 1141, 1098, 1074, 997, 867, 840 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.46 (br d, J = 6.5 Hz, 2H), 7.37—7.29 (m, 3H), 5.48 (s, 1H), 4.75 (dd, J = 4.5, 4.5 Hz, 1H), 4.26 (dd, J = 10.0, 5.0 Hz, 1H), 3.91 (dd, J = 11.0, 3.5 Hz, 1H), 3.86 (dd, J = 10.0, 3.5 Hz, 1H), 3.67 (m, 1H), 3.64 (dd, J = 10.0, 10.0 Hz, 1H), 3.49 (ddd, J = 13.5, 9.0, 4.5 Hz, 1H), 3.43 (dd, J = 10.0, 5.0 Hz, 1H), 3.36 (ddd, J = 12.0, 12.0, 2.0 Hz, 1H), 3.31 (ddd, J = 9.0, 9.0, 4.5 Hz, 1H), 3.20—3.10 (m, 2H), 2.46 (ddd, J = 11.0, 3.5, 3.5 Hz, 1H), 2.23 (dd, J = 11.5, 4.5 Hz, 1H), 2.14 (dd, J = 12.0, 3.0, 1H), 2.02 (m, 1H), 1.91—1.78 (m, 2H), 1.68 (br d, J = 13.0 Hz, 1H), 1.65—1.44 (m, 3H), 1.35 (m, 1H), 1.25 (s, 3H), 0.78 (d, J = 7.5 Hz, 3H), 0.10 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 215.6, 137.4, 129.1, 128.3 (2C), 126.1 (2C), 101.7, 86.4, 85.5, 81.7, 76.6, 75.52, 75.51, 74.2, 73.8, 69.4, 67.6, 45.3, 40.9, 31.1, 30.8, 25.4, 24.5, 22.3, 7.8, 2.5 (3C); HRMS (ESI) calcd for $C_{29}H_{45}O_8Si$ $[(M + H)^+]$ 549.2878, found 549.2878. Data for minor diastereomer: $[\alpha]_D^{25}$ −2.8 (*c* 1.07, $CHCl_3$); IR (film) 3500, 2927, 2854, 1716, 1377, 1251, 1097, 867,

840, 753, 423 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, J = 6.5 Hz, 2H), 7.37—7.29 (m, 3H), 5.48 (s, 1H), 4.26 (dd, J = 10.0, 4.5 Hz, 1H), 4.03 (dd, J = 11.5, 6.5 Hz, 1H), 3.91 (dd, J = 12.0, 4.0 Hz, 1H), 3.84 (dd, J = 10.0, 4.0 Hz, 1H), 3.64 (dd, J = 10.5, 10.5 Hz, 1H), 3.59 (dd, J = 9.5, 5.0 Hz, 1H), 3.49 (ddd, J = 12.0, 9.0, 4.5 Hz, 1H), 3.40—3.27 (m, 2H), 3.27—3.18 (m, 2H), 3.18 (br d, J = 10.0 Hz, 1H), 2.55 (ddd, J = 14.5, 7.0, 7.0 Hz, 1H), 2.23 (dd, J = 11.5, 4.5 Hz, 1H), 2.15 (br d, J = 12.0 Hz, 1H), 2.04 (m, 1H), 1.93 (m, 1H), 1.82 (dd, J = 12.0, 12.0 Hz, 1H), 1.77—1.65 (m, 2H), 1.56 (m, 1H), 1.38—1.28 (m, 2H), 1.26 (s, 3H), 0.84 (d, J = 7.5 Hz, 3H), 0.11 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.0, 137.4, 129.1, 128.3 (2C), 126.1 (2C), 101.7, 88.1, 85.5, 82.5, 79.1, 76.6, 75.6, 74.2, 73.8, 69.4, 67.8, 45.4, 37.6, 31.8, 30.9, 25.4, 25.0, 22.3, 9.7, 2.5 (3C); HRMS (ESI) calcd for $C_{29}H_{44}O_8SiNa$ [$(M + Na)^+$] 571.2698, found 571.2699.

Ester 4. To a solution of α-hydroxy ketone **32** (14.9 mg, 0.0272 mmol) in benzene/MeOH (2:1, v/v, 0.9 mL) cooled to 0 °C was added $Pb(OAc)_4$ (14.6 mg, 0.0330 mmol), and the resultant mixture was stirred at room temperature for 35 min. The reaction was quenched with saturated aqueous $NaHCO_3$ solution, and the resultant mixture was filtered through a pad of Celite. The filtrate was diluted with H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (20% EtOAc/hexanes) gave ester-aldehyde **33** (15.5 mg, ca. 99%), which was immediately used in the next reaction.

To a suspension of $Ph_3P^+CH_3Br^-$ (50 mg, 0.14 mmol) in THF (1 mL) cooled to 0 °C was added NaHMDS (1.0 M solution in THF, 0.10 mL, 0.10 mmol), and the resultant suspension was stirred at 0 °C for 30 min. To this suspension was added a solution of the above ester-aldehyde **33** (15.5 mg) in THF (0.5 mL + 0.2 mL rinse twice), and the resultant mixture was stirred at 0 °C for 35 min. The reaction was quenched with saturated aqueous NH_4Cl solution. The resultant mixture was diluted with EtOAc and washed with brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (10 to 12% EtOAc/hexanes) gave ester **4** (13.2 mg, 84% for the two steps) as a colorless oil. ^1H NMR analysis of this material indicated that it was an approximately 12−13:1 mixture of diastereomers at C25 stereogenic center. Data for **4**: $[\alpha]^{17}_D$ +19.2 (c 1.59, $CHCl_3$); IR (film) 2952, 1752, 1375, 1251, 1142, 1098, 1002, 867, 840, 752, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.46 (br d, J = 6.5 Hz, 2H), 7.38—7.30 (m, 3H), 5.88 (m, 1H), 5.48 (s, 1H), 5.03 (d, J = 12.0 Hz, 1H), 5.02 (dd, J = 16.0, 1.0 Hz, 1H), 4.27 (dd, J = 10.0, 4.5 Hz, 1H), 4.03 (dd, J = 8.5, 5.5 Hz, 1H), 3.88 (dd, J = 11.0, 4.5 Hz, 1H), 3.70 (s, 3H), 3.65 (dd, J = 10.0, 10.0 Hz, 1H), 3.49 (ddd, J = 12.5, 9.0, 4.0 Hz, 1H), 3.31 (ddd, J = 9.5, 9.5, 4.5 Hz, 1H), 3.28—3.21 (m, 2H), 3.16 (br d, J = 8.0 Hz, 1H), 3.03 (dd, J = 8.5, 1.5 Hz, 1H), 2.83 (ddd, J = 14.5, 7.0, 7.0 Hz, 1H), 2.22 (ddd, J = 11.0, 11.0, 4.5 Hz, 2H), 1.93 (m, 1H), 1.87—1.78 (m, 2H), 1.74—1.48 (m, 3H), 1.34—1.20 (m, 2H), 1.25 (s, 3H), 1.10 (d, J = 7.0 Hz, 3H), 0.10 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 139.6, 137.4, 129.1, 128.4 (2C), 126.2 (2C), 115.0, 109.7, 101.8, 85.5, 84.6, 76.7, 75.6, 74.3, 73.8, 69.4, 67.8, 51.7, 45.4, 37.2, 30.9, 29.2, 25.1, 24.3, 22.4, 17.8, 2.5 (3C); HRMS (ESI) calcd for $C_{31}H_{48}O_8SiNa$ [$(M + Na)^+$] 599.3011, found 599.3022.

Alcohol 34. A stock solution of TBAF/AcOH was prepared from TBAF (1.0 M solution in THF, 5.00 mL, 5.00 mmol), AcOH (0.300 mL, 5.25 mmol), and THF (4.7 mL). To a solution of ester **4** (59 mg, 0.10 mmol) in THF (1 mL) was added the above stock solution of TBAF/AcOH (1.00 mL), and the resultant solution was stirred at room temperature for 3 h. The reaction mixture was diluted with EtOAc and washed successively with saturated aqueous NH_4Cl solution, saturated aqueous $NaHCO_3$ solution, and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (20 to 40% EtOAc/hexanes) gave alcohol **34** (55 mg, 100%, ca. 12−13:1 mixture of diastereomers at C25 stereogenic center) as a colorless foam: $[\alpha]^{25}_D$ +28.5 (c 0.70, $CHCl_3$); IR (film) 3483, 2936, 2869, 1752, 1453, 1370, 1093, 1002, 699, 412 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.46 (dd, J = 7.5, 1.0 Hz, 2H), 7.38—7.29 (m, 3H), 5.88 (m, 1H), 5.49 (s, 1H), 5.022 (d, J = 11.5 Hz, 1H), 5.017 (d, J = 16.0 Hz, 1H), 4.27 (dd, J = 11.0, 4.5 Hz, 1H), 4.05 (dd, J = 8.0, 4.5 Hz, 1H), 3.87 (dd, J = 11.0, 4.5 Hz, 1H), 3.70 (s, 3H), 3.64 (dd, J = 10.0, 10.0 Hz, 1H), 3.52 (ddd, J = 13.0, 9.0, 4.0 Hz, 1H), 3.34—3.21 (m, 3H), 3.18 (br d, J = 9.0, 1H), 3.06 (dd, J = 9.0, 1.5 Hz, 1H), 2.82 (ddd, J = 14.0, 7.0, 7.0 Hz, 1H), 2.24—2.16 (m, 2H), 1.96 (m, 1H), 1.86—1.48 (m, 6H), 1.38—1.24 (m, 2H), 1.24 (s, 3H), 1.10 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 139.6, 137.3, 129.1, 128.3 (2C), 126.1 (2C), 115.0, 101.7, 85.1, 84.4, 76.6, 75.4, 74.2, 73.7, 71.1, 69.3, 67.7, 51.8, 45.1, 37.2, 30.6, 29.0, 25.0, 24.3, 21.7, 17.7; HRMS (ESI) calcd for $C_{28}H_{40}O_8Na$ [$(M + Na)^+$] 527.2615, found 527.2604.

Carboxylic Acid 35. To a solution of alcohol **34** (258.4 mg, 0.5127 mmol, ca. 12−13:1 mixture of diastereomers at C25 stereogenic center) in THF/MeOH/H_2O (1:1:1, v/v/v, 4.5 mL) cooled to 0 °C was added $LiOH \cdot H_2O$ (33 mg, 0.79 mmol), and the resultant mixture was stirred vigorously at room temperature for 4 h 15 min. The reaction mixture was cooled to 0 °C and carefully acidified with 1 M aqueous HCl solution (pH ca. 4). The resultant mixture was extracted repeatedly with $CHCl_3$, and the organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (40% EtOAc/hexanes) gave carboxylic acid **35** (222.2 mg, 88%) as a colorless oil. The minor C25-epimer was removed at this stage. Data for **35**: $[\alpha]^{25}_D$ +13.0 (c 0.55, $CHCl_3$); IR (film) 3436, 2935, 2869, 1725, 1455, 1370, 1093, 1006, 758, 680 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.46 (dd, J = 7.5, 1.5 Hz, 2H), 7.38—7.30 (m, 3H), 5.89 (m, 1H), 5.50 (s, 1H), 5.07—5.00 (m, 2H), 4.27 (dd, J = 10.0, 5.0 Hz, 1H), 4.12 (dd, J = 6.0, 6.0 Hz, 1H), 3.89 (br d, J = 11.0 Hz, 1H), 3.67 (dd, J = 10.0, 10.0 Hz, 1H), 3.53 (ddd, J = 13.0, 9.0, 4.5 Hz, 1H), 3.35—3.24 (m, 3H), 3.20 (d, J = 9.0 Hz, 1H), 3.09 (dd, J = 9.0, 2.0 Hz, 1H), 2.71 (ddd, J = 14.0, 7.0, 7.0 Hz, 1H), 2.23—2.14 (m, 2H), 2.04 (m, 1H), 1.84—1.72 (m, 3H), 1.67 (m, 1H), 1.56 (m, 1H), 1.46—1.33 (m, 2H), 1.25 (s, 3H), 1.24 (m, 1H), 1.10 (d, J = 7.0 Hz, 3H) (carboxylic acid proton is missing due to H/D exchange); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8, 139.7, 137.3, 129.1, 128.3 (2C), 126.2 (2C), 115.3, 101.7, 84.9, 84.0, 76.6, 75.4, 74.7, 74.3, 71.2, 69.3, 67.6, 45.0, 37.9, 30.4, 29.4, 25.0, 23.7, 21.6, 17.7; HRMS (ESI) calcd for $C_{27}H_{39}O_8$ [$(M + H)^+$] 491.2639, found 491.2632.

Lactone 3. To a solution of carboxylic acid **35** (214.4 mg, 0.4378 mmol) in THF (8.8 mL) cooled to 0 °C were added Et_3N (0.130 mL, 0.933 mmol) and 2,4,6-$Cl_3C_6H_2COCl$ (0.200 mL, 1.28 mmol). The resultant mixture was stirred at room temperature for 40 min before being diluted with toluene (35 mL). The mixed anhydride solution thus obtained was added dropwise to a solution of DMAP (318 mg, 2.61 mmol) in toluene (44 mL) heated at 100 °C over a period of 6 h. The reaction mixture was cooled to room temperature, diluted with EtOAc, and washed successively with 1 M aqueous HCl solution, saturated aqueous $NaHCO_3$ solution, and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (10 to 30% EtOAc/hexanes) gave lactone **3** (185.9 mg, 90%) as amorphous solid: $[\alpha]^{24}_D$ +40.9 (c 1.00, $CHCl_3$); IR (film) 2932, 2860, 1718, 1455, 1293, 1206, 1070, 1035, 752, 699, 419 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.48—7.44 (m, 2H), 7.38—7.30 (m, 3H), 5.80 (ddd, J = 17.5, 10.5, 8.5 Hz, 1H), 5.51 (s, 1H), 5.08 (d, J = 17.5 Hz, 1H), 5.06 (d, J = 9.0 Hz, 1H), 4.42

(d, J = 4.5 Hz, 1H), 4.29 (dd, J = 10.5, 4.5 Hz, 1H), 3.88 (ddd, J = 11.0, 2.0, 2.0 Hz, 1H), 3.68 (dd, J = 10.5, 10.5 Hz, 1H), 3.56 (ddd, J = 13.5, 9.5, 4.5 Hz, 1H), 3.48—3.33 (m, 3H), 3.25 (ddd, J = 11.0, 11.0, 2.5 Hz, 1H), 3.03 (dd, J = 9.0, 2.0 Hz, 1H), 2.66 (ddd, J = 14.0, 7.0, 7.0 Hz, 1H), 2.41 (dd, J = 12.0, 4.5 Hz, 1H), 2.17 (m, 1H), 2.08 (m, 1H), 1.99 (dd, J = 12.0, 12.0 Hz, 1H), 1.93 (m, 1H), 1.79 (br d, J = 11.5 Hz, 1H), 1.74 (s, 3H), 1.70 (m, 1H), 1.66—1.48 (m, 2H), 1.39 (dddd, J = 12.0, 12.0, 12.0, 5.0 Hz, 1H), 1.08 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 138.8, 137.1, 129.1, 128.3 (2C), 126.1 (2C), 116.1, 101.8, 84.1, 82.7, 82.4, 78.4, 76.5, 76.2, 74.4, 69.1, 67.5, 44.3, 37.7, 30.1, 27.6, 25.1, 24.5, 20.3, 18.1; HRMS (ESI) calcd for $C_{27}H_{37}O_7$ [$(M + H)^+$] 473.2534, found 473.2544.

α-Acetoxy Ether 36. To a solution of lactone **3** (17.8 mg, 0.0377 mmol) in CH_2Cl_2 (1 mL) cooled to −78 °C was added DIBALH (1.04 M solution in hexanes, 0.073 mL, 0.076 mmol), and the resultant solution was stirred at −78 °C for 35 min. To this solution were added pyridine (0.037 mL, 0.46 mmol), Ac_2O (0.043 mL, 0.46 mmol), and a solution of DMAP (60 mg, 0.49 mmol) in CH_2Cl_2 (0.5 mL). The resultant mixture was stirred at −78 °C for 13 h and then allowed to warm to 0 °C over a period of 1 h. The reaction was quenched with saturated aqueous NH_4Cl solution. The resultant mixture was diluted with EtOAc and saturated aqueous potassium sodium tartrate solution and stirred vigorously at room temperature until the layers became clear. The organic layer was separated and washed successively with 1 M aqueous HCl solution, saturated aqueous $NaHCO_3$ solution, and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (20% EtOAc/hexanes) gave α-acetoxy ether **36** (18.9 mg, 97%) as an approximately 5.2:1 mixture of diastereomers: $[\alpha]^{25}_D$ +31.0 (c 1.00, $CHCl_3$); IR (film) 2935, 2867, 1750, 1455, 1368, 1233, 1092, 1013, 698 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, signals for the major diastereomer) δ 7.45 (dd, J = 8.0, 2.0 Hz, 2H), 7.37—7.29 (m, 3H), 5.94 (ddd, J = 17.5, 10.5, 8.5 Hz, 1H), 5.94 (br s, 1H), 5.48 (s, 1H), 5.08—4.98 (m, 2H), 4.27 (dd, J = 10.5, 5.0 Hz, 1H), 3.88 (br d, J = 8.5 Hz, 1H), 3.79 (br s, 1H), 3.68 (dd, J = 10.5, 10.5 Hz, 1H), 3.58 (ddd, J = 11.5, 9.5, 4.5 Hz, 1H), 3.45 (br d, J = 5.0 Hz, 1H), 3.36 (ddd, J = 10.0, 10.0, 4.5 Hz, 1H), 3.25 (ddd, J = 10.5, 10.5, 4.5 Hz, 2H), 3.07 (br d, J = 9.0 Hz, 1H), 2.90 (ddd, J = 14.0, 7.0, 7.0 Hz, 1H), 2.20—1.92 (m, 5H), 2.08 (s, 3H), 1.70—1.50 (m, 4H), 1.45 (m, 1H), 1.39 (s, 3H), 1.09 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$, signals for the major diastereomer) δ 169.3, 140.2, 137.3, 129.0, 128.2 (2C), 126.1 (2C), 115.2, 109.7, 101.7, 91.6, 85.2, 83.6, 76.6, 76.0, 75.3, 74.3, 69.3, 67.8, 42.3, 37.2, 31.4, 29.0, 25.5, 24.2, 21.2, 21.0, 18.1; HRMS (ESI) calcd for $C_{29}H_{40}O_8Na$ [$(M + Na)^+$] 539.2615, found 539.2629.

Diene 2. To a solution of α-acetoxy ether **36** (8.2 mg, 0.016 mmol) in CH_2Cl_2/CH_3CN (1:1, v/v, 1.0 mL) were added a spatula tip of 4 Å molecular sieves (ca. 5 mg) and allyltrimethylsilane (0.5 mL). The resultant mixture was cooled to −40 °C, treated with $BF_3 \cdot OEt_2$ (0.010 mL, 0.081 mmol), and allowed to warm to room temperature over a period of 8.5 h. The reaction was quenched with Et_3N at −40 °C, and the resultant mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (40 to 70% EtOAc/hexanes) gave a diol (5.1 mg, 78%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87 (ddd, J = 17.5, 9.5, 8.0 Hz, 1H), 5.78 (dddd, J = 17.0, 10.0, 7.0, 7.0 Hz, 1H), 5.09—4.94 (m, 4H), 3.90—3.80 (m, 2H), 3.79—3.67 (m, 3H), 3.51 (dd, J = 2.5, 2.5, 1H), 3.23 (ddd, J = 11.0, 11.0, 2.5 Hz, 1H), 3.19 (m, 1H), 3.12—3.04 (m, 2H), 2.66 (ddd, J = 15.0, 7.5, 7.5 Hz, 1H), 2.22—2.13 (m, 2H), 2.12—2.00 (m, 3H), 1.88—1.68 (m, 4H), 1.62—1.46 (m, 3H), 1.31 (dddd, J = 12.0, 12.0, 12.0, 5.0 Hz, 1H), 1.26 (s, 3H), 1.23 (m, 1H), 1.08 (d, J = 7.0 Hz, 3H).

To a solution of the above diol (9.6 mg) in THF (1.2 mL) cooled to 0 °C were added Et_3N (0.048 mL, 0.35 mmol), DMAP (2.8 mg, 0.023 mmol), and Ac_2O (0.020 mL, 0.23 mmol), and the resultant solution was stirred at room temperature for 1 h. The reaction was quenched with MeOH at 0 °C. The resultant mixture was diluted with EtOAc and washed successively with 1 M aqueous HCl solution, saturated aqueous $NaHCO_3$ solution, and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (20% EtOAc/hexanes) gave diene **2** (8.3 mg, 72%) as a colorless oil: $[\alpha]^{25}_D$ +30.1 (c 0.50, $CHCl_3$); IR (film) 2935, 2852, 1744, 1456, 1373, 1240, 1039, 911 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87 (ddd, J = 17.5, 10.5, 8.5 Hz, 1H), 5.77 (dddd, J = 17.0, 10.5, 6.5, 6.5 Hz, 1H), 5.06—4.95 (m, 4H), 4.80 (ddd, J = 10.5, 10.5, 5.0 Hz, 1H), 4.16—4.06 (m, 2H), 3.86 (br d, J = 11.5 Hz, 1H), 3.76 (ddd, J = 6.0, 6.0, 2.0 Hz, 1H), 3.54—3.46 (m, 2H), 3.23 (ddd, J = 11.5, 11.5, 2.5 Hz, 1H), 3.13—3.03 (m, 2H), 2.96 (br d, J = 9.0 Hz, 1H), 2.65 (ddd, J = 14.0, 7.0, 7.0 Hz, 1H), 2.20—2.12 (m, 3H), 2.08 (m, 1H), 2.06 (s, 3H), 2.01 (s, 3H), 1.86 (ddd, J = 12.5, 12.5, 12.5 Hz, 1H), 1.76 (m, 1H), 1.64—1.47 (m, 5H), 1.34 (m, 1H), 1.33 (s, 3H), 1.08 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.0, 139.8, 135.3, 116.9, 115.2, 85.2, 84.8, 80.7, 77.6, 77.2, 75.0, 74.5, 67.8, 67.1, 63.4, 44.3, 41.3, 37.8, 31.2, 26.8, 25.4, 23.5, 21.1, 20.9, 18.5, 15.4; HRMS (ESI) calcd for $C_{27}H_{42}O_8Na$ [$(M + Na)^+$] 517.2772, found 517.2760.

DEFG-Ring Skeleton 1. To a solution of diene **2** (1.8 mg, 0.0036 mmol) in degassed CH_2Cl_2 (1.3 mL) was added a solution of the Grubbs second-generation catalyst (0.9 mg, 0.001 mmol) in degassed CH_2Cl_2 (0.5 mL), and the resultant solution was stirred at 40 °C for 19 h. After being cooled to room temperature, the reaction mixture was treated with Et_3N and stirred at room temperature for 1.5 h. The resultant mixture was concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (15 to 20% EtOAc/hexanes) gave DEFG-ring skeleton **1** (1.7 mg, 100%) as a yellow oil: $[\alpha]^{26}_D$ +59.2 (c 0.18, $CHCl_3$); IR (film) 2931, 1745, 1541, 1241, 1096, 1044, 505, 457 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.67 (ddd, J = 10.8, 10.8, 4.8 Hz, 1H), 5.42 (dd, J = 10.8, 10.8 Hz, 1H), 4.77 (ddd, J = 11.4, 11.4, 5.4 Hz, 1H), 4.12 (dd, J = 12.0, 1.8 Hz, 1H), 4.07 (dd, J = 12.0, 5.4 Hz, 1H), 3.89 (br d, J = 11.4 Hz, 1H), 3.80 (ddd, J = 8.4, 4.2, 4.2 Hz, 1H), 3.45 (m, 1H), 3.22 (ddd, J = 11.4, 11.4, 3.6 Hz, 1H), 3.18—3.10 (m, 2H), 3.10—3.03 (m, 2H), 3.01 (dd, J = 9.6, 4.2 Hz, 1H), 2.78 (ddd, J = 12.3, 12.3, 4.2 Hz, 1H), 2.27 (dd, J = 12.0, 5.4 Hz, 1H), 2.12 (dddd, J = 16.2, 7.2, 7.2, 7.2 Hz, 1H), 2.08 (s, 3H), 2.03 (m, 1H), 2.01 (s, 3H), 1.93 (m, 1H), 1.80—1.68 (m, 2H), 1.65—1.48 (m, 4H), 1.42 (dddd, J = 11.4, 11.4, 11.4, 5.4 Hz, 1H), 1.21 (s, 3H), 1.01 (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 169.9, 134.2, 126.4, 84.8, 84.00, 83.98, 82.0, 77.7, 75.4, 74.4, 68.7, 66.9, 63.3, 44.2, 32.3, 32.2, 32.1, 31.2, 26.2, 24.3, 21.0, 20.9, 15.9, 15.5; HRMS (ESI) calcd for $C_{25}H_{39}O_8$ [$(M + H)^+$] 467.2639, found 467.2645.

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Supporting Information Available: Detailed experimental procedures for compounds not included in the Experimental Section and copies of ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.