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Systemic Study on the Biogenic Pathways of Yezo'otogirins: Total Synthesis and Antitumor Activities of (±)-Yezo'otogirin C and Its Structural Analogues

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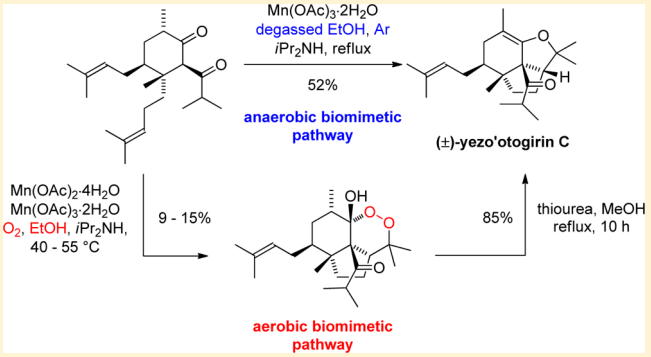
Supporting Information

ABSTRACT: A systematic study of the biomimetic pathways to yezo'otogirin C under aerobic and anaerobic conditions has been investigated, and both are found to be feasible pathways to the natural product depending on the physiological conditions. Because of the lower activation energy, the aerobic process would be more favorable when the in vivo oxygen level is high. In the course of this study, a highly efficient synthetic route to (±)-yezo'otogirin C has been established in four steps (31% overall yield) from a readily available compound without using any protecting groups. The natural product and its structural analogues exhibited antitumor activities against several human cancer cell lines and appeared to arrest cell cycles in different phases.



INTRODUCTION

Plants of the *Hypericum* genus are popular folk medicine to relieve pain, swelling, inflammation, burns, and symptoms of various kinds of neurological disorders.[1] In particular, St. John's wort (*H. perforatum* L.)[2] is a well-known medicinal herb for its anti-inflammatory and antidepression properities.[3] In order to search for plants with high biological values in the *Hypericum* genus, the extracts of many related species have been surveyed for different bioactivities including antiviral, antibacterial, antifungal, antitumor, anti-inflammatory, and antioxidant activities.[4] The tricyclic terpenoid yezo'otogirins A–C (Figure 1), which contain a rare bowl-shape skeleton with four to five stereogenic centers, were isolated from the shoots of *H. yezoense*.[5] Preliminary in vitro assays showed that these natural products are noncytotoxic against L1210 murine leukemia. However, no further study on their biological activities has been reported probably due to the limited supply of these natural products.

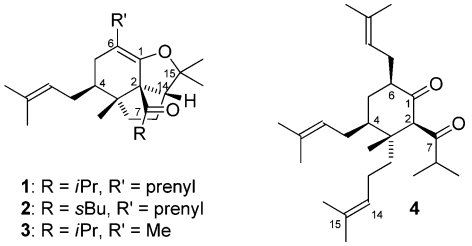


Figure 1. Yezo'otogirins A–C (**1**–**3**) and the coisolate hyperforin derivative (**4**).

The biosynthesis of the yezo'otogirins has not been fully elucidated. A known coisolated hyperforin derivative **4**[6] (Figure 1) was hypothesized as a potential biosynthetic precursor of yezo'otogirin A (**1**).[5] On the basis of this biogenic proposal, two feasible biomimetic pathways to the yezo'otogirins could be considered. As shown in Scheme 1, single-electron oxidative 5-*exo-trig* radical cyclization of precursors **I** could produce the *cis*-hydrindanes of **II**,[7] which could undergo cationic cyclization via another single-electron oxidation to form the tricyclic intermediate (**IV**) under anaerobic conditions (pathway a). Subsequent deprotonation and enolization would afford the yezo'otogirins. An alternative pathway would be the trapping of **II** by a molecular oxygen under aerobic conditions[7,8] (pathway b) forming the peroxy-bridged compounds (**V**). Reduction of **V** followed by elimination could also provide the yezo'otogirins.[8a,9]

In the course of a systematic study on these two potential biogenic pathways to the yezo'otogirins, we have successfully

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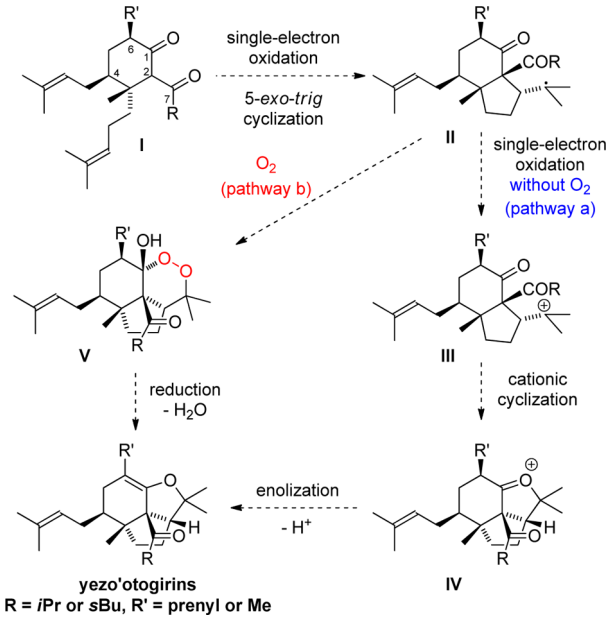
Scheme 1. Potential Biomimetic Pathways for the Yezo'otogirins



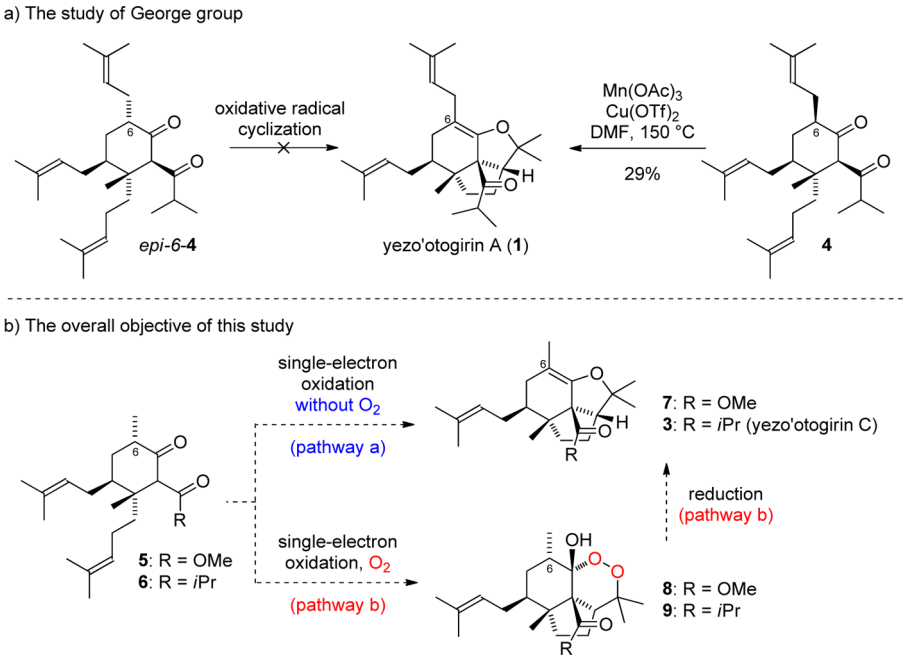
employed β-keto ester **5** as the cyclization precursor of a model study and found that pathway b (oxidative free-radical cyclization under aerobic conditions) is the more favorable synthetic route to the tricyclic core of **7**, which can be converted to (±)-yezo'otogirin C (**3**) in four steps.[10] During the preparation of this manuscript, the group of George reported the attempts of oxidative free-radical cyclization of **4** and *epi*-6-**4**.[11] Interestingly, they found that the stereogenic center at C6 has great influence on the cyclization and only **4** can provide yezo'otogirin A (**1**) in only modest yield under anaerobic conditions at very high reaction temperature (Scheme 2a). We herein report the details of the systematic study on the potential biogenic pathways of yezo'otogirin C (**3**) under aerobic and anaerobic conditions by using β-keto ester **5** as the cyclization precursor of the model study and diketone **6** as the biomimetic cyclization precursor of yezo'otogirin C (**3**) (Scheme 2b).

RESULTS AND DISCUSSION

1. Preparation of β-Keto Ester 5 and Diketone 6. The synthesis of **5** and **6** began with a common starting material (**12**), which was obtained from 1,3-cyclohexanedione in three steps (90% total yield in decagram scales) with known procedures.[12] As shown in Scheme 3, α'-methylation of **12** by LDA/CH_3I gave compound **13** in good yield with excellent diastereoselectivity, which is presumably due to the flat structure of the enolate intermediate and the encumbrance of the prenyl group. β-Keto ester **5** was readily obtained as a mixture of diastereomers by installing the 4-methylpent-3-en-1-yl side chain via conjugation addition and trapping the enolate intermediate with methyl cyanoformate.[13] Diketone **6** was prepared in a similar way via trapping of the enolate intermediate with isobutyraldehyde and oxidation of intermediate **14** (a single diastereomer).[14] Compounds **5** and **6** were prepared efficiently from a known enone (**12**) in only two to three steps with overall yields of 60−72%, respectively. The high diastereoselectivity of the conjugate addition reactions could be attributable to the encumbrance of the prenyl group.

2. Model Study with β-Keto Ester 5 as the Substrate. *a. Anaerobic Oxidative Free-Radical Cyclization of 5.* As β-keto esters are more active substrates for oxidative free-radical cyclizations,[15] β-keto ester **5** was employed as the model cyclization precursor for the biomimetic study. Under anaerobic conditions (Scheme 1, pathway a), oxidative free-radical cyclization of **5** using $Mn(OAc)_3{\cdot}2H_2O$ in acetic acid did not produce the expected cyclization product (**7**) in any significant yield, but a variety of unidentified elimination side products (Table 1, entry 1). A change of solvent to ethanol increased the yield to 13% (entry 2). Encouraged by this result, the effects of other single-electron oxidants[8] including $Cu(OAc)_2{\cdot}H_2O$,

Scheme 2. Study of the George Group and the Overall Objective of This Study



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Scheme 3. Preparation of β-Keto Ester 5 and Diketone 6

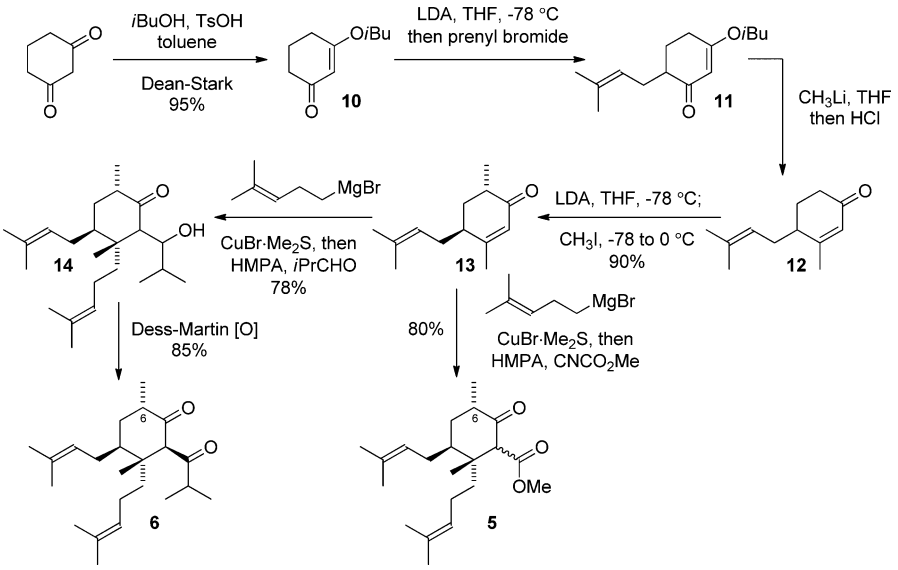
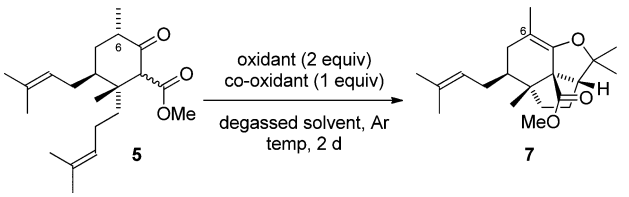


Table 1. Anaerobic Oxidative Free-Radical Cyclization of 5[a]



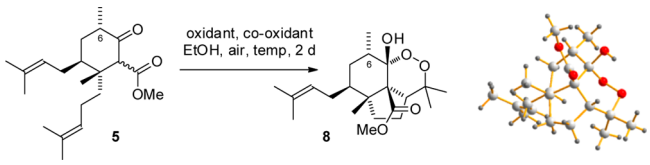
entry	oxidant	co-oxidant	solvent	temp (°C)	yield[b] (%)
1	$Mn(OAc)_3 \cdot 2H_2O$		AcOH	rt	<3
2	$Mn(OAc)_3 \cdot 2H_2O$		EtOH	rt	13
3	$Mn(OAc)_3 \cdot 2H_2O$	$Cu(OAc)_2 \cdot H_2O$	EtOH	rt	22
4	$Mn(OAc)_3 \cdot 2H_2O$	$Cu(OAc)_2 \cdot H_2O$	EtOH	reflux	20

[a]The general procedures for the oxidative free-radical cyclization under anaerobic conditions were followed. [b]Isolated yields (%) after silica gel flash column chromatography.

CAN, and $FeCl_3$ were studied. Unfortunately, these oxidants did not give any of the expected cyclization product (data not shown). The combination of $Mn(OAc)_3 \cdot 2H_2O/Cu(OAc)_2 \cdot H_2O$ in ethanol successfully increased the yield of **7** to 22% (entry 3). However, increasing the reaction temperature resulted in a slightly lower yield (entry 4). Addition of a variety of bases led to slow decomposition of the substrate (**5**) at room temperature probably due to the hydrolysis of the ester moieties (data not shown). The necessity for the formation of the highly strained intermediate (**IV** in Scheme 1) may be the cause of the inefficacy of this cyclization process.[7g]

b. Aerobic Oxidative Free-Radical Cyclization of 5. The aerobic oxidative free-radical cyclization **5** (Scheme 1, pathway b)[7,8] at room temperature were studied with a variety of oxidants. Using $Mn(OAc)_3 \cdot 2H_2O$ in ethanol afforded 20% yield of the peroxy-bridged compound **8** (Table 2, entry 1) together with a number of unidentifiable side products. Compound **8** was found to be a single diastereomer with its structure characterized by X-ray crystallography.[16] Similar to the case in the anaerobic process, other single-electron oxidant, such as

Table 2. Aerobic Oxidative Free-Radical Cyclization of 5[a]



entry	oxidant/co-oxidant	equiv	temp (°C)	yield[b] (%) 8	yield[b] (%) 5
1	$Mn(OAc)_3 \cdot 2H_2O$/–	2.0/–	rt	20	
2	$Cu(OAc)_2 \cdot H_2O$/–	2.0/–	rt	<3	65
3	CAN/–	2.0/–	rt	<3	82
4	$FeCl_3$/–	2.0/–	rt		78
5	$Mn(OAc)_2 \cdot 4H_2O$/–	2.0/–	rt	44	
6	$Mn(OAc)_2 \cdot 4H_2O/Mn(OAc)_3 \cdot 2H_2O$	2.0/0.2	rt	55	
7	$Mn(OAc)_2 \cdot 4H_2O/KMnO_4$	2.0/0.2	rt	36	
8	$Mn(OAc)_2 \cdot 4H_2O/Pb(OAc)_4$	2.0/0.2	rt	31	
9	$Mn(OAc)_2 \cdot 4H_2O/Cu(OAc)_2 \cdot H_2O$	2.0/0.2	rt	35	
10	$Mn(OAc)_2 \cdot 4H_2O/CrO_3$	2.0/0.2	rt	27	
11	$Mn(OAc)_2 \cdot 4H_2O$/CAN	2.0/0.2	rt	42	
12	$Mn(OAc)_2 \cdot 4H_2O/FeCl_3$	2.0/0.2	rt	20	
13	$Mn(OAc)_2 \cdot 4H_2O$/–	1.0/–	rt	38	
14	$Mn(OAc)_2 \cdot 4H_2O$/–	0.5/–	rt	35	
15	$Mn(OAc)_2 \cdot 4H_2O$/–	0.1/–	rt	35	17
16	$Mn(OAc)_2 \cdot 4H_2O$/–	0.1/–	50	35[c]	
17	$Mn(OAc)_2 \cdot 4H_2O/Mn(OAc)_3 \cdot 2H_2O$	0.1/0.1	rt	44	14
18	$Mn(OAc)_2 \cdot 4H_2O/KMnO_4$	0.1/0.1	rt	11	54
19	$Mn(OAc)_2 \cdot 4H_2O/Cu(OAc)_2 \cdot H_2O$	0.1/0.1	rt	38	27
20	$Mn(OAc)_2 \cdot 4H_2O$/CAN	0.1/0.1	rt	27	13

[a]The general procedures for the oxidative free-radical cyclization under aerobic conditions were followed. [b]Isolated yields (%) after silica gel flash column chromatography. [c]Reaction time = 1 d.

$Cu(OAc)_2 \cdot H_2O$, CAN, or $FeCl_3$ only produced a trace amount of desirable products with 65–82% of recovered starting materials (entries 2–4). Interestingly, $Mn(OAc)_2 \cdot 4H_2O$ (which generated Mn(III) in the presence of oxygen) afforded 44%

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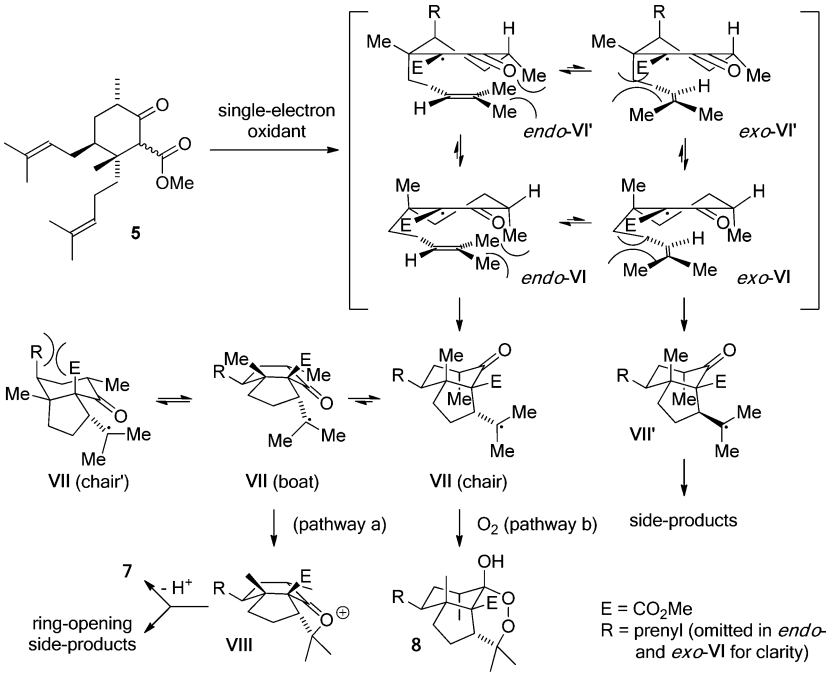
Scheme 4. Analysis of the Molecular Conformation of the Reaction Intermediates



yield of **8** (entry 5). With this encouraging result in hand, the effects of a variety of co-oxidants with $Mn(OAc)_2{\cdot}4H_2O$ were investigated (entry 6−12).[8b,c] The optimal results were obtained by using Kurosawa and Nishino's combination of oxidants ($Mn(OAc)_2{\cdot}4H_2O/Mn(OAc)_3{\cdot}2H_2O$),[8b] which afforded the 55% yield of **8** (entry 6), and the formation of compound **7** was not observed.

After a survey of the effects on the oxidant loading, we found that using a catalytic amount (0.1 equiv) of $Mn(OAc)_2{\cdot}4H_2O$ only caused a minor drop in the yield of **8** (35−38%, entries 13−15) and the cyclization rate. The use of 0.1 equiv of $Mn(OAc)_2{\cdot}4H_2O$ resulted in incomplete reaction (entry 15). Although the reaction can be forced to completion at alleviated temperature (50 °C) for 2 days, the isolated yield cannot be improved because of the thermal instability of **8** (entry 16). Addition of 0.1 equiv of co-oxidant ($Mn(OAc)_3{\cdot}2H_2O$ or $Cu(OAc)_2{\cdot}H_2O$) afforded **8** in 44 and 38% yield (entries 17 and 19, respectively).

The results of the above model study could be rationalized by detail analysis of the molecular conformation of the reaction intermediates in both biogenic pathways. The radical intermediate generated from single-electron oxidation of **5** could adopt four possible half-chair conformations prior to the 5-*exo*-*trig* cyclization (Scheme 4). According to the Beckwith's transition state model,[17] *endo*-**VI**/**VI**′ are considered more favorable than *exo*-**VI**/**VI**′. It could also be rationalized by the encumbrance generated by the manganese (not shown for clarity) complexed to both the ketone and the ester carbonyls with the *gem*-substituted alkene in *exo*-**VI**/**VI**′. Cyclization of the less congested *endo*-**VI**′ requires a higher energy twist-boat transition state, while the *endo*-**VI** could cyclize via a generally more favorable chairlike transition state, but a strong 1,3-diaxial interaction between the axial methyl and the incoming alkene may disfavor this reaction path. Thus, both *endo*-**VI** and **VI**′ could undergo cyclization and lead to **VII** (chair). Cyclization of *exo*-**VI**/**VI**′ gives radical **VII**′ containing *β*-*i*-Pr and leads to various side products as cyclization with the ketone cannot take place. Radical **VII** (chair) possesses the proper conformation for cyclization with a molecular oxygen to form the peroxy-bridged compound **8**. On the other hand, **VII** (chair) needs to undergo conformational changes to the less stable **VII** (boat) or **VII** (chair) in order to cyclize with the ketone. The resultant tricyclic cation **VIII** is highly strained and can undergo either deprotonation/enolization to afford **7** or ring opening to give a variety of side products.

Although the experimental results indicated that the anaerobic process for the formation of the tricyclic compound **7** from **5** is a possible pathway, this conformational analysis suggested that the activation energy of the anaerobic process (pathway a) is much higher than that of the aerobic process (pathway b) due to the conformational changes and the formation of highly strained intermediate during the reaction. Thus, the aerobic process (pathway b) for the formation of the peroxy-bridged compound (**8**) is the more favorable pathway under aerobic conditions. This is also supported by the fact that the formation of **7** was not observed under the aerobic conditions.

3. Conversion of 8 to (±)-Yezo'otogirin C (3). With **8** in hand, a number of conditions for reduction of the peroxy-bridge moiety were studied.[8a,9] As shown in Table 3, PPh_3 in refluxing CH_2Cl_2 or zinc dust in hot ethanol did not give any expected product (entries 1 and 2). Switching the solvent to acetic acid with zinc dust resulted in 28% yield of **7** (entry 3). Using $CuCl/CH_3CN$ and hydrogenation with Pd/C provided only a modest yield of **7** (entries 4 and 5). Thiourea in acetic acid at 60 °C gave **7** in 68% yield (entry 6). Switching the solvent to methanol improved the yield to 80%. The conditions were finally optimized by using thiourea in refluxing methanol,[8a] which afforded **7** in 92% yield (entry 8). The NMR data of **7** from the reduction of **8** are identical to those for the compound obtained from **5** under anaerobic conditions.

Compound **7** was expected to be transformed to (±)-yezo'otogirin C (**3**) in one pot upon treatment of *i*-PrMgBr or *i*-PrLi.[18] However, when excess *i*-PrMgBr was used

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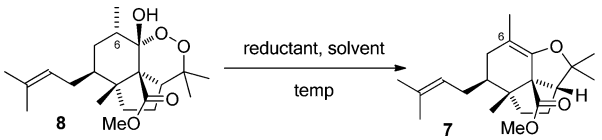
Table 3. Reduction of Peroxy-Bridged Compound 8



entry	reductant	solvent	temp (°C)	yield[a] (%)
1	PPh_3	CH_2Cl_2	reflux	
2	Zn	EtOH	60	
3	Zn	AcOH	60	28
4	CuCl	CH_3CN	rt	40
5	Pd/C, H_2	MeOH	70	47
6	thiourea	AcOH	70	68
7	thiourea	MeOH	70	80
8	thiourea	MeOH	reflux	92

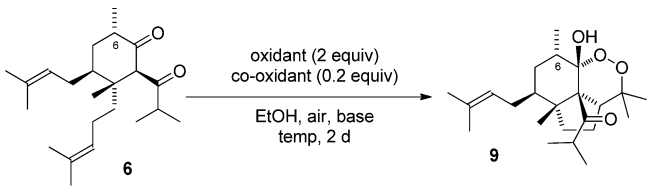
[a]Isolated yield (%) after silica gel flash column chromatography.

at room temperature, no reaction was observed. Elevation of the reaction temperature resulted in the decomposition of compound **7**. Also, no product was formed by using 1 equiv of *i*-PrLi at −78 °C to ambient temperature. Instead, decarboxylation of **7** was observed when a large amount of *i*-PrLi was used and resulted in 50−80% yield of **15** at 0 °C or room temperature (Scheme 5). Switching to basic conditions for hydrolysis of the methyl ester or Lewis acidic conditions for direct conversion of the methyl ester to the corresponding Weinreb amide also resulted in no reaction. Eventually, (±)-yezo'otogirin C (**3**) was obtained via DIBAL reduction of **7** followed by TPAP/NMO oxidation, *i*-PrLi addition to the resultant aldehyde (**17**), and a subsequent Dess−Martin oxidation. NMR spectral data of the final product are identical to those reported in the literature.[5]

The formation of the decarboxylation side-product **15** would be resulted from either a double *i*-PrLi addition/retro-aldol sequence or a Krapcho-type decarboxylation.[19] To study the mechanism of this unusual decarboxylation process, (±)-yezo'otogirin C (**3**) was submitted to a large excess of *i*-PrLi from 0 °C to room temperature. Interestingly, (±)-yezo'otogirin C (**3**) was found to be stable with *i*-PrLi. This result indicated that double addition of *i*-PrLi to the ester moiety of **7** is not feasible, and the decarboxylation side product **15** would be most likely formed via the Krapcho-type decarboxylation mechanism.

4. Systematic Study of the Biomimetic Pathways to (±)-Yezo'otogirin C (3). *a. Aerobic Oxidative Free-Radical Cyclization of 6.* Encouraged by the results of the model study, the optimal conditions of the aerobic oxidative free-radical cyclization of **5** were employed for study of the cyclization of **6**.[7,8] However, single-electron oxidation of diketone **6** turned out to be much more difficult than that of *β*-keto ester **5**. After a survey of different oxidative conditions, we found that cyclization of **6** at room temperature led to no reaction or only a trace amount of the peroxy-bridged compound **9** (Table 4, entries 1−6). The reaction proceeded much faster under high reaction temperature (55 °C) with an amine additive (entry 7). Although further increasing the reaction temperature enhanced the cyclization rate, the peroxy-bridged product **9** was found to

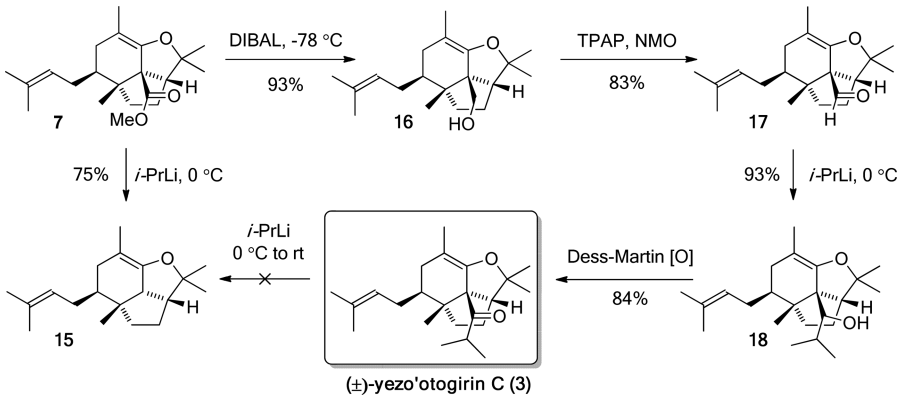
Table 4. Aerobic Oxidative Free-Radical Cyclization of 6[a]



entry	oxidant	co-oxidant	base	temp (°C)	yield[c] (%)
1	$Mn(OAc)_3 \cdot 2H_2O$			rt	<3
2	$Mn(OAc)_2 \cdot 4H_2O$			rt	<3
3	$Cu(OAc)_2 \cdot H_2O$			rt	*d*
4	CAN			rt	*d*
5	$FeCl_3$			rt	*d*
6	$Mn(OAc)_2 \cdot 4H_2O$		base[b]	rt	<3
7	$Mn(OAc)_2 \cdot 4H_2O$		base[b]	55	3−7
8	$Mn(OAc)_2 \cdot 4H_2O$		base[b]	reflux	*e*
9	$Mn(OAc)_2 \cdot 4H_2O$	$Mn(OAc)_3 \cdot 2H_2O$	iPr_2NH	rt	<3
10	$Mn(OAc)_2 \cdot 4H_2O$	$Mn(OAc)_3 \cdot 2H_2O$	iPr_2NH	40	15
11	$Mn(OAc)_2 \cdot 4H_2O$	$Mn(OAc)_3 \cdot 2H_2O$	iPr_2NH	55	9
12	$Mn(OAc)_2 \cdot 4H_2O$	$Mn(OAc)_3 \cdot 2H_2O$	iPr_2NH	reflux	*e*

[a]The general procedures for oxidative free-radical cyclization under aerobic conditions were followed. [b]Base = pyridine, DBU, Et_3N, $nBuNH_2$, piperidine, Et_2NH or iPr_2NH. [c]Isolated yield (%) after silica gel flash column chromatography. [d]No reaction. [e]Compound **6** was consumed.

Scheme 5. Conversion of 7 to (±)-Yezo'otogirin C (3)



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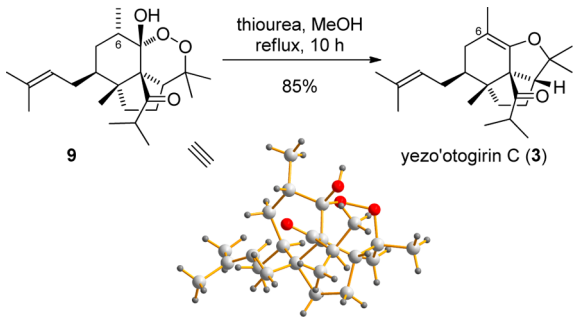
be unstable under refluxing temperature and resulted in decomposition. The reaction temperature was then optimized by balancing the rates for the formation of **9** and its decomposition (entry 9−12). Although cyclization of **6** can be proceeded at a temperature as low as 40 °C, the reaction afforded only 15% yield (a single diastereomer) due to the decomposition of **9** (entry 10). There was no formation of the natural product under these conditions. The structure of **9** was characterized by X-ray crystallography.[16] Despite the disappointing results, (±)-yezo'otogirin C (**3**) was eventually obtained via reacting **9** with thiourea in refluxing methanol (Scheme 6).

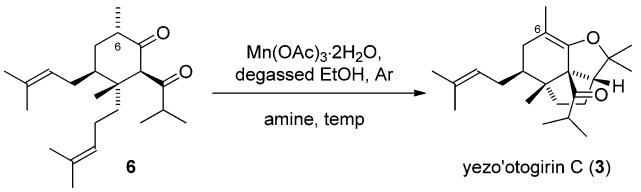
Scheme 6. Conversion of 9 to (±)-Yezo'otogirin C (3)



b. Anaerobic Oxidative Free-Radical Cyclization of ***6****.* On the basis of the above results, both amine additives and high reaction temperature are beneficial to the radical initiation of diketone **6**. The amine base would also promote the late-stage deprotonation and enolization of the tricyclic cation intermediate (**IV**, Scheme 1). Thus, the effects of a variety of amines under different reaction temperature were studied under anaerobic conditions. As shown in Table 5, $Mn(OAc)_3 \cdot 2H_2O$ in ethanol provided only a trace amount of (±)-yezo'otogirin C (**3**) at room temperature (entry 1). Addition of a base resulted in similar results (entry 2). Increasing the reaction temperature generally enhanced the rate and the yields of the reaction, which is consistent with the results under aerobic conditions. With pyridine as the base in refluxing ethanol, the reaction gave 6% yield of (±)-yezo'otogirin C (**3**) as a single diastereomer (entry 3). Switching to a stronger base, such as DBU or triethylamine, greatly increased the yields to 22 and 30% respectively (entries 4 and 5). Both primary and secondary amines are able to enhance the efficiency of the cyclization process and afforded 34−52% yield of the natural product (entry 6−9). Lowering the reaction temperature to 40 °C (the optimal temperature for the aerobic pathway) led to very poor efficiency (entry 10). Addition of a co-oxidant (entry 11) or Brønsted acid (entry 12−15) did not improve the yields of the reaction. Finally, the conditions were optimized by using $Mn(OAc)_3 \cdot 2H_2O$ with diisopropylamine in refluxing ethanol (entry 9), which afforded 52% yield of (±)-yezo'otogirin C (**3**) as a single diastereomer. The NMR data of (±)-yezo'otogirin C (**3**) from diketone **6** are identical to those in the model study and the literature.[5]

Contrary to the results of the model study, diketone **6** preferentially underwent oxidative free-radical cyclization under anaerobic conditions and directly led to the natural product (**3**) in one pot with good yields. This result suggested that the anaerobic process is a possible biogenic pathway of (±)-yezo'otogirin C (**3**) when the in vivo oxygen level is low.

Table 5. Anaerobic Oxidative Free-Radical Cyclization of Diketone 6.[a]



entry	additive	base	temp	yield[c] (%)
1			rt	<3
2		base[b]	rt	<3
3		pyridine	reflux	6
4		DBU	reflux	22
5		Et_3N	reflux	30
6		*n*-$BuNH_2$	reflux	34
7		piperidine	reflux	34
8		Et_2NH	reflux	47
9		*i*-Pr_2NH	reflux	52
10		*i*-Pr_2NH	40 °C	<3
11	$Cu(OAc)_2 \cdot H_2O$	*i*-Pr_2NH	reflux	36
12	AcOH	*i*-Pr_2NH	reflux	8
13	Cl_2HCCO_2H	*i*-Pr_2NH	reflux	20
14	TsOH	*i*-Pr_2NH	reflux	10
15	L-proline		reflux	10

[a]The general procedures for the oxidative free-radical cyclization under anaerobic conditions were followed. [b]Base = pyridine, DBU, Et_3N, *n*$BuNH_2$, piperidine, Et_2NH or *i*Pr_2NH. [c]Isolated yield (%) after silica gel flash column chromatography.

However, the initiation of the anaerobic process required refluxing temperature of ethanol (78 °C), which is an unusual physiological condition. On the other hand, the temperature required for the aerobic process is much lower (40 °C), and the anaerobic process cannot be initiated at this temperature. These results suggested that the aerobic process would be the more favorable pathway when the in vivo oxygen level is high. This is also supported by the fact that the direct formation of the natural product was not observed under the aerobic conditions.

c. Study on the Effects of Bases and Temperature. During the study on the cyclization of diketone **6** under the anaerobic conditions, epimerization of the stereogenic center at C6 of diketone **6** was observed. Therefore, the effects of the base and reaction temperature on the stereogenic center at C6 were studied. As showed in Figure 2, diisopropylamine at room temperature did not lead to epimerization at C6 (entry 1). On the other hand, the stereogenic center at C6 epimerized slowly upon heating in ethanol (entry 2). Using *i*-Pr_2NEt in refluxing ethanol also led to the formation of *epi*-6-**6** (entry 3−5). The ratio of **6**/*epi*-6-**6** obtained after 3 days of heating with *i*-Pr_2NEt (entry 5) is similar to that without *i*-Pr_2NEt (entry 2). In the presence of $Mn(OAc)_3 \cdot 2H_2O$, the disappearance rate of *epi*-6-**6** is much higher than that of **6** (entry 6). The results of this study indicated that the C6 stereogenic center of **6** would be epimerized under the high reaction temperature conditions, and *epi*-6-**6** is a more active substrate for the anaerobic process. This observation is also consistent with that reported by the group of George.[11] The *epi*-6-**6** is more likely to be the bioprecursor of (±)-yezo'otogirin C (**3**) for its higher reactivity and the same configuration at C6 of the hyperforin derivative **4**.

5. Antitumor Activities of (±)-Yezo'otogirin C (3) and Its Structural analogues (7−9 and 15). The antitumor activity of (±)-yezo'otogirin C (**3**) and its structural analogues

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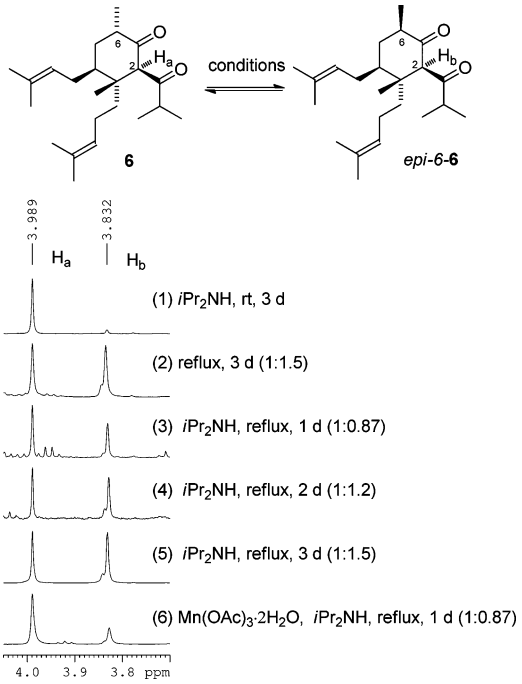

Figure 2. NMR study on the effects of base and temperature.

(7–9 and 15) toward a number of human cancer cell lines was studied by MTT assays. All four compounds exhibited cell inhibition activities against human cervical, liver, and gastric cancer cells (Table 6). For gastric cancer MGc80-3,

Table 6. IC_{50} of the Cell Growth Inhibitory Effects of (±)-Yezo'otogirin C (3) and Its Structural Analogues (7–9 and 15) by MTT Assays[a]

	IC_{50} (μM)				
cell lines	3	7	8	9	15
HeLa	32.82	88.33	32.15	310.64	28.88
SMMC-7721	20.41	57.78	34.22	101.87	23.85
MGc80–3	9.54	27.15	9.98	62.88	12.05
cell arrested at	G2	G2	G1	G1, S	G2

[a]The general procedures for the biological assays were followed.

(±)-yezo'otogirin C (3) and the peroxy-bridged ester analogue (8) exhibited good antitumor activity (IC_{50} = 9.54 and 9.98 μM, respectively). The deisobutrylated analogue (15) also showed good antitumor activity (IC_{50} = 12.05 μM). The ester analogue (7) showed a moderate activity, and its peroxy-bridged analogue (9) showed the lowest IC_{50} value. In the flow cytometry cell cycle analyses, (±)-yezo'otogirin C (3), its ester analogue (7), and the deisobutrylated analogue (15) mainly caused G2 phase arrest of cell cycles. In particular, the deisobutrylated analogue (15) induced a 6.6-fold increment of G2 cell cycle arrestment (43.79% to 6.62%) in a concentration-dependent manner (Table S1, Supporting Information) and also a massive cell death in 60 μM. On the other hand, peroxy-bridged compounds 8 and 9 inhibit cell grow mainly in G1 phase in a concentration-dependent manner (from 10 to 50 μM), and 9 showed inhibition in S phase in high concentration (60 μM).

CONCLUSION

In summary, the aerobic and anaerobic biomimetic pathways of (±)-yezo'otogirin C (3) have been studied, and both are found to be possible pathways to the natural product depending on the physiological conditions. The aerobic process would be the more favorable biogenic pathway when the in vivo oxygen level is high due to its lower activation energy. In the course of this study, a highly efficient synthetic route to (±)-yezo'otogirin C has been established in four steps (31% overall yield) from a readily available compound (12) without using any protecting groups. An asymmetric synthesis could be readily obtained by employing enantiomerically enriched 12.[14,20] The natural product (3) and its analogues (7–9 and 15) showed anticancer activity against several human cancer cell lines with IC_{50} values up to 9.54 μM. These compounds appeared to arrest cell cycles in different phases. We are currently preparing a library of structural analogues using this biomimetic strategy for a detail structure–activity relationship study, and exploring the potential applications of this highly convergent oxidative free-radical cyclization strategy on other classes of natural products, such as picrotoxanes.[21]

EXPERIMENTAL SECTION

General Information for Synthesis. Unless otherwise stated, all air- and water-sensitive reactions were performed under inert atmosphere (N_2 or Ar) and anhydrous conditions with dry solvents. Reactions were monitored by thin-layer chromatography (TLC) performed on 0.25 mm thick silica gel plates (60 F_{254}) under 254 nm ultraviolet irradiation or via *p*-anisaldehyde staining (150 mL ethanol of 5.00 mL of concentrated H_2SO_4, 1.50 mL of glacial HOAc, and 3.70 mL of anisaldehyde). Flash column chromatography was carried out on silica gel (200–300 mesh). All commercial chemicals were used without further purification. Anhydrous THF was distilled over powdered sodium and benzophenone. Anhydrous toluene was distilled from powdered sodium. Anhydrous CH_3CN and CH_2Cl_2 were distilled over calcium hydride. ^1H NMR and ^{13}C NMR spectra were recorded using a 300, 400, or 500 MHz spectrometer. The NOESY experiments were carried out using a 400 or 500 MHz spectrometer. The multiplicities of ^1H NMR were designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). High-resolution mass spectra were obtained on an electrospray ionization time-of-flight (ESI-TOF) mass spectrometer. Melting points were uncorrected and obtained with a micromelting point meter. Crystallographic data were achieved using a single-crystal X-ray diffractometer. IR spectra (shown as wavenumbers, cm^{-1}) were measured using an FTIR spectrometer.

General Information for Biological Assays. Human cervical cancer cell HeLa were maintained in DMEM medium; Human gastric cancer cell MGc80-3 and human liver cancer cell SMMC-7721 were cultured in RPMI-1640 medium; both medium were supplemented with 10% fetal bovine serum and antibiotics. For cell viability assay (MTT), cells were seeded in 96-well plates and were exponentially growth. Then cells were dosed with chemicals and treated for 36 h. Live cell population were analyzed using the MTT assay, by addition of equal amounts of 3-(4,5-dimethylthiazol-2-yl)-2 and 5-diphenyltetrazolium bromide (0.5 mg/mL) for 4 h to produce formazan in living cells. The reaction products were dissolved by addition of dimethyl sulfoxide, and the absorbance of the solutions was measured in microplate reader. Reactions were performed in triplicate for each concentration of the chemicals. For flow cytometry assay (cell cycle), cells were harvested by trypsinization, centrifuged, rinsed in 1× PBS twice, and then fixed in cold 70% ethanol for 120 min. Before analysis, propidium iodide (20 μg/mL) and RNaseA (0.1 mg/mL) were added to the cells for nucleic acid staining and RNA digestion. Samples were analyzed in a flow cytometry analyzer.

3-Methyl-4-(3-methylbut-2-en-1-yl)cyclohex-2-enone (12).[12] To a stirred solution of cyclohexane-1,3-dione (50.00 g, 0.45 mol) in freshly

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distilled toluene (1 L) under argon were added 2-methylpropan-1-ol (99.11 g, 1.34 mol) and TsOH (0.38 g, 2.23 mmol). The solution was heated under reflux, and water was removed with a Dean−Stark trap for 4 h. The solution was then concentrated under reduced pressure. Silica gel column chromatography (EtOAc/hexanes 1:5) of the crude mixture afforded a yellow oil (71.25 g, 0.42 mol) as the product (**10**). The THF (200 mL) solution of compound **10** (71.25 g, 0.42 mol) was added dropwise to LDA (233.26 mL, 0.47 mol) in THF (1 L) at −78 °C. After 30 min, 1-bromo-3-methylbut-2-ene (69.53 g, 0.47 mol) was added slowly at −78 °C. The solution was stirred at −78 °C for 0.5 h and 0 °C until the full consumption of starting **10**. A saturated aqueous NH_4Cl solution was added to quench the reaction. Ethyl acetate (500 mL × 3) was used to extract the aqueous layer. The combined organic solution was treated by $MgSO_4$, filtered, and concentrated. Purification of the crude mixture by flash chromatography (EtOAc/hexanes 1:20) gave a yellow oil (100 g, 0.42 mol) as the product (**11**). MeLi (0.39 L, 0.51 mmol) was added dropwise to a solution of **11** (100 g, 0.42 mol) in THF (1 L) at 0 °C. The resulting solution was stirred at ambient temperature for 1 h and then treated with a 4 N aqueous HCl (160 mL) slowly at 0 °C. After the reaction was stirred at ambient temperature for 30 min, ethyl acetate (500 mL × 3) was used to extract the aqueous layer. The combined organic solution was washed using aqueous $NaHCO_3$ solution and aqueous NaCl solution and dried by $MgSO_4$. After filtration and concentration under reduced pressure, silica gel flash column chromatography (EtOAc/hexanes 1:20) of the crude mixture gave a yellow oil (72.2 g, 0.41 mol, 90% in three steps from cyclohexane-1,3-dione) as the product. **12**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.84 (s, 1H), 5.10−5.12 (m, 1H), 2.35−2.47 (m, 1H), 2.21−2.31 (m, 3H), 2.10−2.20 (m, 1H), 1.94−2.05 (m, 4H), 1.78−1.90 (m, 1H), 1.72 (s, 3H), 1.62 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.5, 165.7, 133.9, 126.9, 121.8, 39.9, 34.0, 29.7, 26.5, 25.8, 23.0, 17.8; IR (neat, cm^{-1}): 3032, 2964, 2930, 2873, 1685, 1625, 1458, 1374, 1223, 874; HRMS (ESI/$[M + H]^+$) calcd for $C_{12}H_{19}O$ 179.1436, found 179.1428.

*3,6-Dimethyl-4-(3-methylbut-2-en-1-yl)cyclohex-2-enone (**13**).* *n*-BuLi (13.88 mL, 33.30 mmol) was added dropwise to a stirred THF (100 mL) solution of *i*-Pr_2NH (4.88 mL, 34.69 mmol) at 0 °C. The resulting mixture was allowed to stir at room temperature for 30 min and then cooled to −78 °C. A THF (20 mL) solution of compound **12** (4.94 g, 27.75 mmol) was added slowly to the flask at −78 °C. After the solution was stirred for 0.5 h, MeI (3.46 mL, 55.51 mmol) was added slowly to the solution at −78 °C. Then the mixture was allowed to stir at −78 °C for 0.5 h and 0 °C until the full consumption of **16**. A saturated aqueous NH_4Cl solution was added to quench the reaction. Ethyl acetate (100 mL × 3) was used to extract the aqueous layer. The combined organic solution was washed using aqueous NaCl solution and treated with $MgSO_4$. After filtration and concentration under reduced pressure, flash chromatography (EtOAc/hexanes 1:20) of the crude mixture afforded a yellow oil (4.53 g, 23.59 mmol, 85%) as the desired product. **13**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.79 (s, 1H), 5.15 (t, J = 6.5 Hz, 1H), 2.49−2.42 (m, 1H), 2.31−2.17 (m, 3H), 1.97−1.93 (m, 4H), 1.78−1.72 (m, 4H), 1.63 (s, 3H), 1.10 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.7, 164.4, 133.9, 126.2, 122.3, 40.1, 36.2, 34.9, 29.8, 25.7, 22.8, 17.8, 15.4; IR (neat, cm^{-1}): 3027, 2970, 2927, 2872, 1674, 1628, 1448, 1374, 1213, 878, 854; HRMS (ESI/$[M + H]^+$) calcd for $C_{13}H_{21}O$ 193.1592, found 193.1588.

*Methyl 2,5-Dimethyl-3-(3-methylbut-2-en-1-yl)-2-(4-methylpent-3-en-1-yl)-6-oxocyclohexanecarboxylate (**5**).* To a stirred suspension of mashed magnesium (2.5 g, 104.2 mmol) and iodine (0.1 g) in THF (80 mL) under argon was added a portion (3 mL) of a solution of 5-bromo-2-methyl-2-pentene (10.2 g, 62.5 mmol) in THF (15 mL). The suspension was heated gently to initiate the reaction. Then the rest of the solution was added slowly to the suspension. The resulting suspension was refluxed for 1.5 h and cooled to ambient temperature. This solution was transferred slowly to a THF (10 mL) solution of CuBr·Me_2S (1.1 g, 5.2 mmol) at −20 °C via a gastight syringe. After the the solution was stirred for 0.5 h, a THF (10 mL) solution of **13** (4 g, 20.84 mmol) was added dropwise to the mixture at −20 °C. After the solution was stirred at −20 °C for 20 min, HMPA (12.8 mL, 72.9 mmol) and methyl cyanoformate (5.8 mL, 72.9 mmol) were added dropwise to the flask at −20 °C. The resulting solution was stirred at −20 °C for 30 min and 0 °C overnight. Saturated NaCl aqueous solution was added to quench the reaction, and ethyl acetate (100 mL × 3) was used to extract the aqueous layer. The combined organic solvent was treated with $MgSO_4$. After filtration and concentration under reduced pressure, flash chromatography (EtOAc/hexanes = 1:50) of the crude mixture provided a colorless oil (5.57 g, 16.67 mmol, 80%) as the desired product. **5** (a mixture a high enolizable β-keto esters): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.17−5.03 (m, 2H), 3.70−3.56 (m, 3H), 2.67−2.42 (m, 1H), 2.56−2.22 (m, 1H), 2.16−2.10 (m, 1H), 2.02−1.83 (m, 3H), 1.81−1.73 (m, 5H), 1.68−1.65 (m, 5H), 1.63−1.60 (m, 4H), 1.57−1.49 (m, 2H), 1.32−1.12 (m, 3H), 1.09−1.02 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 210.6, 207.3, 169.3, 169.0, 133.1, 132.9, 131.8, 131.4, 124.2, 123.6, 123.4, 122.8, 63.0, 60.4, 51.5, 44.5, 44.5, 42.9, 39.9, 39.7, 38.3, 37.2, 34.5, 33.6, 33.2, 27.0, 25.8, 25.8, 25.7, 25.6, 22.7, 21.6, 21.3, 18.2, 17.9, 17.9, 17.6, 17.4, 14.4; IR (neat, cm-1): 3457, 2966, 2927, 2881, 1755, 1713, 1633, 1597, 1436, 1378, 1339, 1213, 1133, 1007, 846; HRMS (ESI/$[M + H]^+$) calcd for $C_{21}H_{35}O_3$ 335.2586, found 335.2581.

*1-Hydroxy-2-methylpropyl)-3,6-dimethyl-4-(3-methylbut-2-en-1-yl)-3-(4-methylpent-3-en-1-yl)cyclohexanone (**14**) (Single Diastereomer).* To a stirred mixture of mashed magnesium (0.25 g, 10.42 mmol) and iodine (0.01 g) in THF (12 mL) under argon was added a portion (0.3 mL) of a solution of 5-bromo-2-methyl-2-pentene (1.36 g, 8.3 mmol) in dried THF (2 mL). The suspension was heated gently to initiate the reaction. Then the rest of the solution was added slowly to the suspension. The resulting suspension was refluxed for 1.5 h and cooled to ambient temperature. This solution was transferred to a THF (5 mL) solution of CuBr·Me_2S (54 mg, 0.26 mmol) at −20 °C slowly using a gastight syringe. After the solution was stirred for 30 min, **13** (1 g, 5.2 mmol) dissolved in THF (2 mL) was added slowly to the flask at −20 °C. After the mixture was stirred at −20 °C for 20 min, a solution of isobutyraldehyde (0.61 mL, 6.8 mmol) in THF (2 mL) was added to the suspension slowly at −78 °C. The suspension was stirred at −78 °C until full consumption of compound **13**. A saturated NH_4Cl aqueous solution was added to quench the reaction, and ethyl acetate (20 mL × 3) was used to extract the aqueous layer. The combined organic solvent was treated by $MgSO_4$. After filtration and concentration under reduced pressure, flash chromatography (EtOAc/hexanes = 1:40) of the crude mixture provided a colorless oil (1.4 g, 4.1 mmol, 78%). **14**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.08−5.07 (m, 2H), 3.72 (d, J = 11.0 Hz, 1H), 3.36 (dd, J = 11.0, 9.0 Hz, 1H), 2.88 (s, 1H), 2.50−2.45 (m, 1H), 2.18−2.14 (m, 1H), 2.07−1.94 (m, 2H), 1.84−1.77 (m, 2H), 1.76−1.74 (m, 1H), 1.73−1.68 (m, 8H), 1.66−1.63 (m, 1H), 1.62 (s, 3H), 1.61 (s, 3H), 1.57−1.55 (m, 1H), 1.23 (d, J = 7.5 Hz, 3H), 1.01 (d, J = 7.0 Hz, 3H), 0.98 (s, 3H), 0.85 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 221.7, 132.6, 131.5, 123.8, 123.2, 75.7, 51.0, 46.5, 45.6, 37.8, 36.8, 35.0, 34.2, 27.6, 25.8, 25.7, 21.2, 20.2, 19.8, 18.3, 17.9, 17.7; IR (neat, cm-1): 3520, 2981, 2930, 2868, 1689, 1456, 1385, 1201, 1121, 994, 832; HRMS (ESI/$[M + H]^+$) calcd for $C_{23}H_{41}O_2$ 349.3107, found 349.3089.

*2-Isobutyryl-3,6-dimethyl-4-(3-methylbut-2-en-1-yl)-3-(4-methylpent-3-en-1-yl)cyclohexanone (**6**) (Single Diastereomer).* Compound **14** (1.4 g, 4.0 mmol) and $NaHCO_3$ (1.35 g, 16.1 mmol) were dissolved in CH_2Cl_2 (30 mL), followed by the addition of Dess−Martin periodinate (2.1 g, 4.8 mmol) at 0 °C. The resulting suspension was stirred at ambient temperature until TLC analysis showed the full consumption of **14**. Diethyl ether (30 mL) and saturated $Na_2S_2O_3$ aqueous solution were added sequentially to the reaction at 0 °C to quench the reaction. After the solution turned clear, ethyl acetate (30 mL × 3) was used to extract the aqueous layer. The combined organic solvent was treated by $MgSO_4$. After filtration and concentration under reduced pressure, flash chromatography (EtOAc/hexanes = 1:70) of the crude mixture provided a colorless oil (1.18 g, 3.4 mmol, 85%). **6**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.07−5.06 (m, 1H), 5.00 (t, J = 6.5 Hz, 1H), 4.00 (s, 1H), 2.68−2.61 (m, 1H), 2.55−2.47 (m, 1H), 2.16−2.13 (m, 1H), 2.07−2.01 (m, 1H), 1.89−1.81 (m, 3H), 1.75−1.70 (m, 5H), 1.70 (s, 3H), 1.62 (s, 3H), 1.59 (s, 3H), 1.46−1.42 (m, 2H), 1.24 (d, J = 7.5 Hz, 3H), 1.08−1.05 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.8, 211.2, 132.8, 131.7, 123.6, 123.1,

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64.2, 45.3, 43.2, 43.0, 38.0, 37.8, 33.8, 26.8, 25.8, 25.6, 22.0, 18.0, 18.0, 17.9, 17.8, 17.6, 17.5; IR (neat, cm^{-1}): 3466, 2971, 2928, 2869, 1726, 1699, 1460, 1380, 1092, 1048, 836; HRMS (ESI/$[M + H]^+$) calcd for $C_{23}H_{39}O_2$ 347.2950, found 347.2938.

General Procedure for Anaerobic Oxidative Free-Radical Cyclization Reaction. To a stirred solution of **5** or **6** (∼50 mg, 0.15 mmol) in EtOH (10 mL) was added the appropriate amount and combination of oxidant (2 equiv), co-oxidant (1 equiv), base (1 equiv), and acid (1 equiv). The mixture was then frozen and degassed under vacuum (× 3). The solution was stirred at the selected reaction temperature under argon for 2 days or until TLC analysis showed the full consumption of **5** or **6**. A saturated NH_4Cl aqueous solution was added to quench the reaction, and ethyl acetate (10 mL × 3) was used to extract the aqueous layer. The combined organic solvent was treated by $MgSO_4$. After filtration and concentration under reduced pressure, the product was obtained by flash chromatography (EtOAc/hexanes 1:100 to 1:40) of the crude mixture.

*Methyl 2,2,4a,7-Tetramethyl-5-(3-methylbut-2-en-1-yl)-2,2a,2a1,3,4,4a,5,6-octahydroindeno[7,1-bc]furan-2a1-carboxylate (**7**) from **5**.* The general procedure for anaerobic conditions was followed with **5** (47 mg, 0.14 mmol) as the substrate in EtOH (10 mL) using $Mn(OAc)_3·2H_2O$ (151 mg, 0.56 mmol) and $Cu(OAc)_2·H_2O$ (56 mg, 0.28 mmol) at room temperature for 2 days. A white solid (10 mg, 0.03 mmol, 22%) was obtained by flash chromatography (EtOAc/hexanes = 1:40). **7**: mp = 65.7−66.8 °C; ^1H NMR (400 MHz, $CDCl_3$) δ5.10 (t, *J* = 6.8 Hz, 1H), 3.67 (s, 3H), 3.02 (dd, *J* = 10.8, 7.2 Hz,1H), 1.98−1.75 (m, 5H), 1.71−1.69 (m, 4H), 1.67 (s, 3H), 1.64−1.49 (m,5H), 1.44−1.36(m, 1H), 1.26 (s, 3H), 1.21(s, 3H), 0.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 147.7, 132.2, 124.4, 107.0, 67.0, 56.2, 51.9, 48.7, 46.9, 41.1, 31.5, 29.7, 29.3, 25.8, 25.8, 25.2, 19.6, 17.8, 16.0; IR (neat, cm^{-1}): 2971, 2932, 2859, 1721, 1449, 1381, 1237, 1160, 1024, 870, 730; HRMS (ESI/$[M + H]^+$) calcd for $C_{21}H_{33}O_3$ 333.2430, found 333.2425.

General Procedure for Aerobic Oxidative Free-Radical Cyclization Reaction. To a stirred solution of **5** or **6** (∼50 mg, 0.15 mmol) in a solvent (10 mL) was added the appropriate amount and combination of oxidant (2 equiv), co-oxidant (0.2 equiv), and base (1 equiv). The mixture was stirred at the appropriate temperature under oxygen for 2 days or until full consumption of **5** or **6**. A saturated NaCl aqueous solution was added to quench the reaction, and ethyl acetate (10 mL × 3) was used to extract the aqueous layer. The combined organic solvent was treated with $MgSO_4$. After filtration and concentration under reduced pressure, the product was obtained by flash chromatography (EtOAc/hexanes 1:20) of the crude mixture.

*Methyl 8a-Hydroxy-3,3,5a,8-tetramethyl-6-(3-methylbut-2-en-1-yl)decahydroindeno[7,1-cd][1,2]dioxine-3a1-carboxylate (**8**).* The general procedures under aerobic conditions were followed with **5** (48 mg, 0.14 mmol) as the substrate using $Mn(OAc)_2·4H_2O$ (73 mg, 0.30 mmol) and $Mn(OAc)_3·2H_2O$ (8 mg, 0.03 mmol) at room temperature for 2 days. A white solid (30 mg, 0.08 mmol, 55%) was achieved as the desired compound **8** by flash chromatography (EtOAc/hexanes 1:10). **8**: mp = 101.5−102.2 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 5.10 (t, *J* = 6.4 Hz, 1H), 3.82 (s, 1H), 3.69 (s,3H), 3.17 (d, *J* = 7.2 Hz, 1H), 2.99−2.90 (m, 1H), 2.07−1.87 (m, 4H), 1.81−1.70 (m,5H), 1.60(s, 3H), 1.55−1.47 (m, 2H), 1.44(s, 3H), 1.39−1.31 (m, 1H), 1.27 (s, 3H), 1.02 (d, *J* = 7.2 Hz, 3H), 0.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ174.5, 132.3, 123.7, 102.1, 80.5, 64.0, 51.3, 50.0, 48.9, 42.1, 41.3, 32.2, 31.9, 30.4, 29.6, 26.7, 25.8, 24.1, 21.9, 17.8, 14.9; IR (neat, cm^{-1}): 3471, 2966, 2939, 2881, 1725, 1638, 1453, 1385, 1237, 1082; HRMS (ESI/$[M + Na]^+$) calcd for $C_{21}H_{34}NaO_5$ 389.2304, found 389.2298.

*Methyl 2,2,4a,7-Tetramethyl-5-(3-methylbut-2-en-1-yl)-2,2a,2a1,3,4,4a,5,6-octahydroindeno[7,1-bc]furan-2a1-carboxylate (**7**) from **8**.* Thiourea (11 mg, 0.14 mmol) was added in one portion to a solution of **8** (44 mg, 0.12 mmol) in MeOH (2 mL). The resulting mixture was heated under refluxed and stirred for 10 h. Concentration and flash chromatography (EtOAc/hexanes = 1:40) of the crude afforded a white solid (36 mg, 0.105 mmol, 92%) as the product. **7**: The characterization data of the white solid are identical to those for the compound prepared from **5**.

*2,2,4a,7-Tetramethyl-5-(3-methylbut-2-en-1-yl)-2,2a,2a1,3,4,4a,5,6-octahydroindeno[7,1-bc]furan (**15**).* To a stirred THF (2 mL) solution of compound **7** (106 mg, 0.32 mmol) was added isopropyllithium (0.96 mL, 0.96 mmol) dropwise at 0 °C under argon. The resulting solution was stirred at 0 °C for 10 min, followed by the addition of NH_4Cl aqueous solution. Ethyl acetate (5 mL × 3) was used to extract the aqueous layer. The combined organic solvent was treated by $MgSO_4$. After filtration and concentration under reduced pressure, silica gel column chromatography (EtOAc/hexanes 1:50) of the crude mixture provided a pale yellow oil (70 mg, 0.25 mmol, 80%) as the product. **15**: ^1H NMR (500 MHz, $CDCl_3$) δ 5.13 (t, *J* = 7.3 Hz, 1H), 2.57 (d, *J* = 6.5 Hz, 1H), 2.28 (m, 1H), 2.05−2.02 (m, 1H), 1.90−1.83 (m, 1H), 1.78−1.67 (m, 7H), 1.60−1.56 (m, 6H), 1.53−1.47 (m, 1H), 1.34 (s, 3H), 1.30−1.24 (m, 4H), 1.22−1.17 (m, 1H), 1.00 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.0, 131.7, 124.9, 100.6, 84.2, 54.1, 52.2, 46.5, 44.3, 39.8, 32.2, 30.1, 29.2, 26.1, 25.8, 24.5, 17.8, 15.6, 15.5; IR (neat, cm^{-1}): 2971, 2932, 2884, 2855, 1718, 1645, 1446, 1378, 1269, 1162, 1099, 909, 866, 749; HRMS (ESI/$[M + H]^+$) calcd for $C_{19}H_{31}O$ 275.2375, found 275.2372.

*2,2,4a,7-Tetramethyl-5-(3-methylbut-2-en-1-yl)-2,2a,2a1,3,4,4a,5,6-octahydroindeno[7,1-bc]furan-2a1-yl)methanol (**16**).* DIBAL (0.83 mL, 1.2 M in toluene, 0.99 mmol) was added slowly to a stirred solution of compound **7** (55 mg, 0.17 mmol) in DCM (4 mL) at −78 °C under argon. The reaction was stirred at −78 °C for 2 h, followed by the slow addition of methanol (0.5 mL) and NaCl aqueous solution (10 mL) at 0 °C. After being stirred at ambient temperature for 30 min, ethyl acetate (10 mL × 3) was used to extract the aqueous layer. The combined organic solvent was treated by $MgSO_4$. After filtration and concentration under reduced pressure, silica gel column chromatography (EtOAc/hexanes 1:50) of the crude mixture afforded a white solid (49 mg, 0.16 mmol, 93%) as the product. **16**: ^1H NMR (500 MHz, $CDCl_3$) δ 5.11 (t, *J* = 6.5 Hz, 1H), 3.70−3.62 (m, 2H), 2.21 (t, *J* = 8.0 Hz,1H), 2.02−1.99 (m, 1H), 1.88−1.84 (m, 2H), 1.82−1.76 (m, 1H), 1.74−1.70 (m,4H), 1.65 (s, 3H), 1.60 (s, 3H), 1.58−1.55 (m, 2H), 1.41 (s, 3H), 1.39−1.33 (m, 1H), 1.27−1.23 (m, 1H), 1.19 (s, 3H), 1.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.60, 131.95, 124.87, 107.62, 83.96, 65.43, 61.27, 55.09, 46.50, 46.05, 41.68, 31.85, 30.23, 29.76, 25.79, 25.50, 25.36, 18.32, 17.83, 16.00; IR (neat, cm^{-1}): 3461, 2976, 2927, 2859, 1718, 1645, 1451, 1381, 1259, 1164, 1101, 1053, 861; HRMS (ESI/$[M + H]^+$) calcd for $C_{20}H_{33}O_2$ 305.2481, found 305.2473.

*2,2,4a,7-Tetramethyl-5-(3-methylbut-2-en-1-yl)-2,2a,2a1,3,4,4a,5,6-octahydroindeno[7,1-bc]furan-2a1-carbaldehyde (**17**).* Compound **16** (50 mg, 0.16 mmol), NMO (77 mg, 0.008 mmol), and 4 Å molecular sieves (20 mg) were dissolved in DCM (10 mL) followed by the addition of TPAP (130 mg, 0.32 mmol) at ambient temperature. The reaction was stirred at ambient temperature for 3 h and then treated with brine (10 mL) at 0 °C. Ethyl acetate (10 mL × 3) was used to extract the aqueous layer. The combined organic solvent was treated by $MgSO_4$. After filtration and concentration under reduced pressure, flash chromatography (EtOAc/hexanes 1:50) of the crude mixture afforded a colorless oil (41 mg, 0.14 mmol, 83%) as the desired product. **17**: ^1H NMR (500 MHz, $CDCl_3$) δ 9.36 (s, 1H), 5.11 (t, *J* = 7.0 Hz, 1H), 2.94 (dd, *J* = 10.0, 7.5 Hz, 1H), 2.00−1.96 (m,1H), 1.94−1.91 (m, 1H), 1.87−1.78 (m, 3H), 1.72 (s, 3H), 1.71 (s,3H), 1.69−1.65 (m, 1H), 1.63−1.54 (m, 4H), 1.40−1.34 (m, 1H), 1.26−1.24 (m, 4H), 1.22 (s, 3H), 0.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.9, 146.7, 132.5, 124.0, 107.2, 84.9, 72.4, 51.6, 49.0, 45.6, 41.2, 32.3, 29.6, 29.3, 25.8, 25.3, 25.3, 19.9, 17.8, 16.2; IR (neat, cm^{-1}): 2971, 2932, 2859, 2711, 1715, 1446, 1383, 1262, 1169, 1101, 866, 722; HRMS (ESI/$[M + H]^+$) calcd for $C_{20}H_{31}O_2$ 303.2324, found 303.2315.

*2-Methyl-1-(2,2,4a,7-tetramethyl-5-(3-methylbut-2-en-1-yl)-2,2a,2a1,3,4,4a,5,6-octahydroindeno[7,1-bc]furan-2a1-yl)propan-1-ol (**18**).* Isopropyllithium (0.25 mL, 1 M in hexanes, 0.25 mmol) was added dropwise at 0 °C to the stirred solution of **17** (15 mg, 0.05 mmol) in THF (2.5 mL) under an argon atmosphere. The solution was stirred at 0 °C for 5 min, followed by treatment using saturated NaCl aqueous solution (5 mL). Ethyl acetate (10 mL × 3) was used to

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extract the aqueous layer, and the combined organic solution was treated by $MgSO_4$.

After filtration and concentration under reduced pressure, flash chromatography (EtOAc/hexanes 1:100) of the crude mixture afforded provided a pale yellow oil (16 mg, 0.047 mmol, 93%) as the desired compound **18**. **18** (mixture of diastereomers): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.11 (t, J = 7.0 Hz, 1H), 3.49 (dd, J = 10.5, 1.5 Hz, 1H), 2.76 (t, J = 7.5 Hz, 1H), 2.11−2.07 (m, 1H), 1.97−1.78 (m, 6H), 1.72 (s, 3H), 1.61 (s,3H), 1.60 (s, 3H), 1.55−1.51 (m, 2H), 1.47 (s, 3H), 1.44−1.39 (m, 1H), 1.13 (s, 3H), 1.01 (s, 3H), 0.99 (d, J = 7.0 Hz, 3H), 0.88 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.4, 132.0, 124.7, 109.7, 84.3, 79.7, 63.9, 55.9, 47.0, 44.4, 43.7, 32.9, 31.0, 30.3, 28.2, 25.8, 25.0, 24.7, 23.0, 19.3, 17.8, 16.3, 16.0; IR (neat, cm^{-1}): 3491, 2971, 2923, 2869, 1721, 1643, 1451, 1381, 1242, 1099, 997, 853; HRMS (ESI/[M + H]$^+$) calcd for $C_{23}H_{39}O_2$ 347.2950, found 347.2941.

*(±)-Yezo'otogirin C (**3**) from **18**.* To the stirred solution of **18** (16 mg, 0.046 mmol) in DCM (3 mL) were added $NaHCO_3$ (16 mg, 0.185 mmol) and Dess−-Martin periodinate (23 mg, 0.055 mmol) in one portion at 0 °C. The reaction was stirred at ambient temperature for 1 h, followed by treatment with saturated $Na_2S_2O_3$ aqueous solution. Ethyl acetate (10 mL × 3) was used to extract the aqueous layer. The combined organic solution was treated by $MgSO_4$. After filtration and concentration under reduced pressure, flash chromatography (EtOAc/hexanes 1:100) of the crude mixture provided a colorless oil (13 mg, 0.039 mmol, 84%) as the natural product. (±)-Yezo'otogirin C (**3**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.12 (t, J = 6.8 Hz, 1H), 3.19 (t, J = 9.6 Hz, 1H), 2.96 (m, 1H),1.99−1.92(m, 2H), 1.89−1.87 (m, 1H), 1.85−1.80 (m, 1H), 1.78−1.76 (m,1H), 1.73 (s, 3H), 1.71 (s, 3H), 1.61 (s, 3H), 1.56−1.52 (m, 2H), 1.40−1.35 (m, 1H), 1.24−1.21 (m, 1H), 1.18 (s, 3H), 1.15 (s, 3H), 1.03 (d, J = 2.4 Hz, 3H), 1.01 (d, J = 2.8 Hz, 3H), 0.75 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.5, 149.4, 132.5, 124.2, 107.5, 83.4, 73.6, 54.9, 48.5, 47.0, 41.5, 37.9, 32.8, 29.6, 29.5, 25.9, 25.4, 25.4, 21.5, 19.6, 18.3, 17.8, 16.3; IR (neat, cm^{-1}): 3466, 2971, 2932, 2876, 2850, 1715, 1694, 1643, 1468, 1451, 1381, 1259, 1162, 1104, 1026, 802; HRMS (ESI/[M + H]$^+$) calcd for $C_{23}H_{37}O_2$ 345.2794, found 345.2788.

*1-(8a-Hydroxy-3,3,5a,8-tetramethyl-6-(3-methylbut-2-en-1-yl)-decahydroindeno[7,1-cd][1,2]dioxin-3a1-yl)-2-methylpropan-1-one (**9**).* The general procedure under aerobic conditions was followed with **6** (50 mg, 0.14 mmol) as the substrate using $Mn(OAc)_2{\cdot}4H_2O$ (71 mg, 0.29 mmol), $Mn(OAc)_3{\cdot}2H_2O$ (8 mg, 0.03 mmol), and i-Pr_2NH (0.02 mL, 0.15 mmol) at 40 °C. After purification using flash chromatography, a white solid (9 mg, 0.019 mmol, 15%) was achieved as the desired product. **9**: mp = 88.1−90.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.09 (t, J = 7.2 Hz, 1H), 3.52 (s, 1H), 3.09−3.00 (m, 2H), 2.96−2.86 (m, 1H), 2.05−2.00 (m, 1H), 1.97−1.89 (m, 2H), 1.88−1.80 (m, 1H), 1.76−1.70 (m, 4H), 1.63−1.59(m, 5H), 1.50−1.43 (m, 4H), 1.36−1.29 (m, 4H), 1.15 (d, J = 6.8 Hz, 3H), 1.07 (d, J = 6.4 Hz, 3H), 0.98 (d, J = 7.2 Hz, 3H), 0.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.9, 132.2, 123.6, 102.8, 80.3, 67.9, 50.8, 47.6, 40.6, 40.5, 36.7, 32.2, 31.9, 30.2, 29.9, 26.9, 25.8, 24.5, 23.1, 21.2, 19.5, 17.8, 14.6; IR (neat, cm^{-1}) 3493, 2968, 2930, 2875, 1709, 1645, 1463, 1379, 1265, 1100, 1041, 805; HRMS (ESI/[M + Na]$^+$) calcd for $C_{23}H_{38}NaO_4$ 401.2668, found 401.2659.

*(±)-Yezo'otogirin C (**3**) from **9**.* Thiourea (11 mg, 0.14 mmol) was added in one portion to the stirred MeOH (2 mL) solution of **9** (44 mg, 0.12 mmol). The solution was refluxed for 10 h. Concentration and flash chromatography (EtOAc/hexanes = 1:40) of the crude mixture afforded a white solid (33 mg, 0.97 mmol, 85%) as the natural product. **(±)-yezo'otogirin C (3)**: The characterization data of the white solid are identical to those for the compound prepared from **18**.

*(±)-Yezo'otogirin C (**3**) from **6**.* The general procedures under anaerobic conditions was followed using **6** (50 mg, 0.14 mmol) as the substrate with $Mn(OAc)_3{\cdot}2H_2O$ (78 mg, 0.29 mmol) and i-Pr_2NH (0.02 mL, 0.15 mL), and a colorless oil (25 mg, 0.073 mmol, 52%) was obtained as the product.**(±)-yezo'otogirin C (3)**: The characterization data of the white solid are identical to those for the compound prepared from **19**.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

X-ray structures of compounds **8** and **9**, MTT assays on human cancer cells and flow cytometry cell cycle analysis of Hela cells for compound **3**, **7**−**9**, and **15**, and ^{1}H NMR and ^{13}C NMR spectroscopic data for the new 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 interest.

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