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Total Synthesis of (+)-Cytosporolide A via a Biomimetic Hetero-Diels–Alder Reaction

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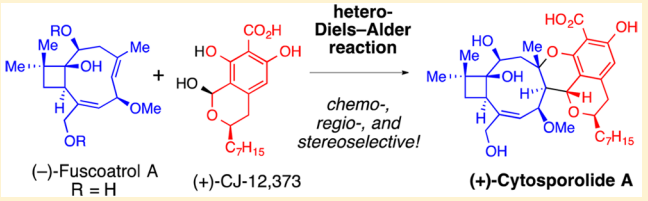
Supporting Information

ABSTRACT: The first total synthesis of (+)-cytosporolide A was achieved by a biomimetic hetero-Diels–Alder reaction of (−)-fuscoatrol A with *o*-quinone methide generated from (+)-CJ-12,373. The dienophile, highly oxygenated caryophyllene sesquiterpenoid (−)-fuscoatrol A, was synthesized from the synthetic intermediate in our previous total synthesis of (+)-pestalotiopsin A. The *o*-quinone methide precursor, isochroman carboxylic acid (+)-CJ-12,373, was synthesized through a Kolbe–Schmitt reaction and an oxa-Pictet–Spengler reaction. The hetero-Diels–Alder reaction of these two compounds proceeded with complete chemo-, regio-, and stereoselectivity to produce the complicated pentacyclic ring system of the cytosporolide skeleton. This total synthesis unambiguously demonstrates that natural cytosporolide A has the structure previously suggested.



INTRODUCTION

(+)-Cytosporolides A–C were isolated by Che and co-workers in 2010 from the fungus *Cytospora* sp., which was found in a soil sample collected on the Qinghai-Tibetan plateau at high altitude.[1] These compounds showed antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pneumoniae*. Based on NMR experiments, the structure of (+)-cytosporolide A was originally assigned as **1** (Figure 1A), which features an unusual peroxylactone skeleton. The absolute stereochemistry was determined by a combination of NOESY data and CD spectra. Later, a structural revision of this natural product was suggested by Spence and George after they re-evaluated the NMR data.[2] Revised structure **2** consists of a complicated pentacyclic ring system containing an oxygenated caryophyllene skeleton connected to a substituted isochroman ring. A biogenetic study indicated that (+)-cytosporolide A (**2**) is derived from a hetero-Diels–Alder reaction between (−)-fuscoatrol A (**3**) and *o*-quinone methide intermediate **5** generated from (+)-CJ-12,373 (**4**) (Figure 1B).[3] Putative precursor **3** is a known caryophyllene sesquiterpenoid isolated from the marine fungus *Humicola fuscoatra*,[4] and was also isolated along with cytosporolides from *Cytospora* sp. Its structure has been established by X-ray diffraction data and NMR spectroscopy. The other precursor, isochroman carboxylic acid **4**, has been previously isolated from *Penicillium* sp. as a topoisomerase II inhibitor.[5] These known compounds could be coupled to create the intricate structure of cytosporolides in nature.

Related natural products, (−)-guajadial (**6**) and (+)-psidial A (**7**), have been isolated from the leaves of *Psidium guajava* L. (guava) (Figure 2A).[6,7] Similar to cytosporolides, it has been suggested that natural products **6** and **7** are formed biosynthetically via a hetero-Diels–Alder reaction of (−)-*β*-caryophyllene (**8**) with an *o*-quinone methide. Figure 2B shows the two main conformers (*βα*:*ββ* in a 3:1 ratio) of caryophyllene **8** at room temperature.[8] The assigned configurations at C-4 and C-5 of **6** and **7** indicate that the biosynthetic Diels–Alder reaction involves the more stable *βα* conformer of **8**. In addition, in the total synthesis of **6** and **7** by Lee and co-workers, the stereochemical outcome of the Diels–Alder reaction of **8** was controlled by the conformation of **8**.[9] However, the configurations at C-8 and C-9 of (+)-cytosporolide A (**2**) were the opposite of those of the corresponding stereogenic centers in **6** and **7**. The precursor of **2**, (−)-fuscoatrol A (**3**) occurs in solution as two conformers, favoring the *βα* conformer (*βα*:*ββ* in a 3:1 ratio).[4] Nevertheless, the proposed biosynthesis of **2** involves cycloaddition of the *ββ* conformer. We wondered what factors cause *o*-quinone methide to react with the less stable *ββ* conformer of **3** in the Diels–Alder reaction.

Considering these results and our previous interest in highly oxygenated caryophyllene sesquiterpenoids,[10] we investigated the Diels–Alder reaction forming (+)-cytosporolide A (**2**).[11] Here, we describe the first total synthesis of **2** via a biomimetic hetero-Diels–Alder reaction and thereby unambiguously establish the structure of natural (+)-cytosporolide A.

RESULTS AND DISCUSSION

Our retrosynthetic analysis of (+)-cytosporolide A (**2**) is shown in Scheme 1. Guided by the putative biosynthesis of **2**, we expected that target molecule **2** would be assembled by a hetero-Diels–Alder reaction using (−)-fuscoatrol A (**3**) and (+)-CJ-12,373 (**4**) as substrates. This approach seemed

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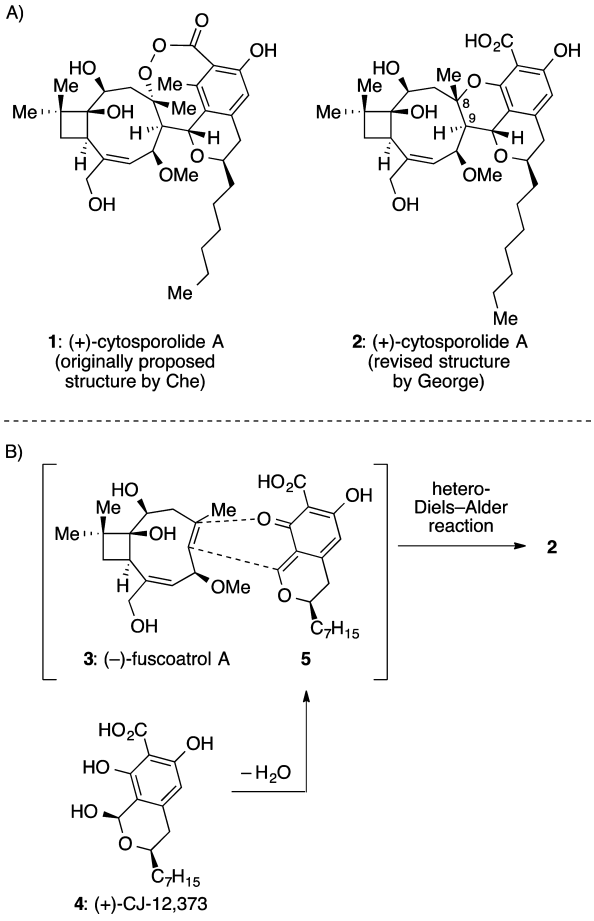


Figure 1. (A) Originally proposed and revised structures of (+)-cytosporolide A. (B) Proposed biosynthesis of (+)-cytosporolide A.

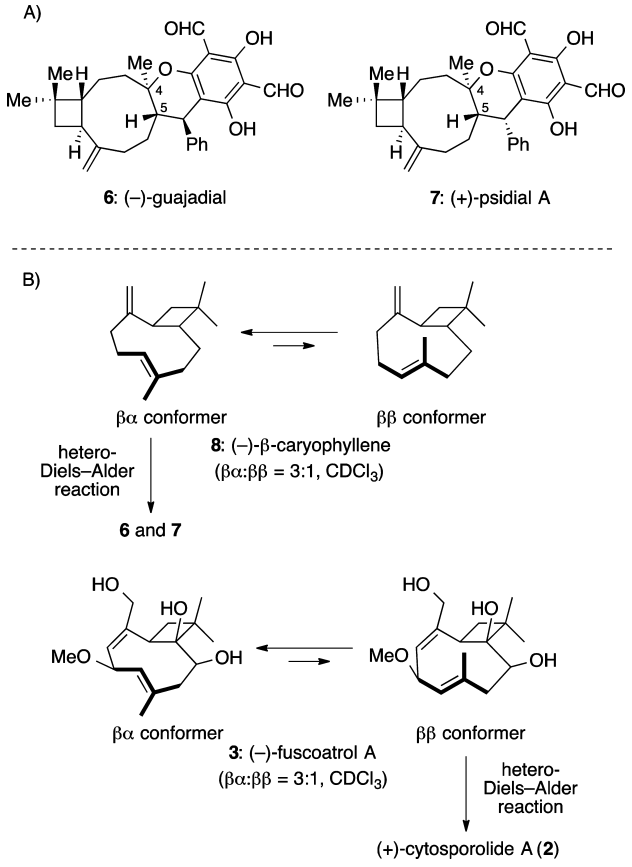
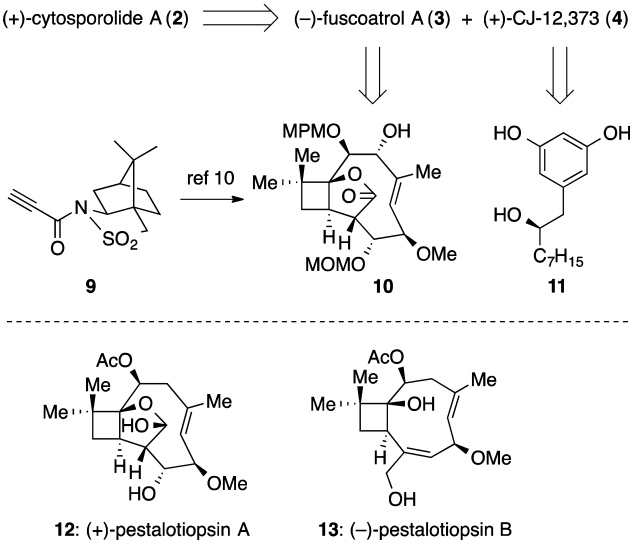


Figure 2. (A) Related natural products (−)-guajadial and (+)-psidial A. (B) Conformations of (−)-*β*-caryophyllene and (−)-fuscoatrol A.

Scheme 1. Retrosynthetic Analysis of (+)-Cytosporolide A (2)



particularly attractive because the Diels−Alder reaction can construct the complicated cytosporolide skeleton in a single step. For the synthesis of **3**, we decided to use key synthetic intermediate **10** from our previous total synthesis of (+)-pestalotiopsin A (**12**).[10] Pestalotiopsins A and B (**12** and **13**) are caryophyllene sesquiterpenoids with structures very similar to **3**.[12] The intriguing structure of pestalotiopsins has attracted the attention of synthetic chemists.[13] Although the formation of the highly oxygenated and strained (*E*)-cyclononene skeleton was difficult, we solved the problem by using an intramolecular Nozaki−Hiyama−Kishi (NHK) reaction and eventually achieved the first and, to date, only total synthesis of **12**. Highly enantioenriched **10** (>95% *ee*) can be obtained from *N*-propioloyl Oppolzer's camphorsultam **9** according to our reliable method and the structure of **10** consists of a highly oxygenated caryophyllene skeleton; therefore, compound **10** was a suitable synthetic intermediate for pre-target **3**. The other pre-target, **4**, would be synthesized from chiral alcohol **11** according to a modified procedure used for the synthesis of (+)-pulvilloric acid, a natural product related to **4**.[14] We envisaged that the *o*-quinone methide intermediate would be generated from **4** or its equivalent by a well-established method.[15]

We first explored the synthesis of (−)-fuscoatrol A (**3**) from our previous synthetic intermediate **10**, which was synthesized through a [2+2] cycloaddition of *N*-propioloyl camphorsultam **9** with ketene acetal and an efficient intramolecular NHK reaction.[10] Mesylation of **10** followed by cleavage of the (4-methoxyphenyl)methyl ether afforded allylic mesyl ester **14** (Scheme 2). Treatment of **14** with a combination of $Pd_2(dba)_3$/*n*-Bu_3P and $NaBH_4$ provided deoxygenated product **15**.[16] Although ring-opening reactions of the *γ*-lactone ring in **15**, accompanied by *β*-elimination of the methoxymethyl ether, were initially attempted, they gave unsatisfactory results. Therefore, lactone **15** was converted into lactol **16** by

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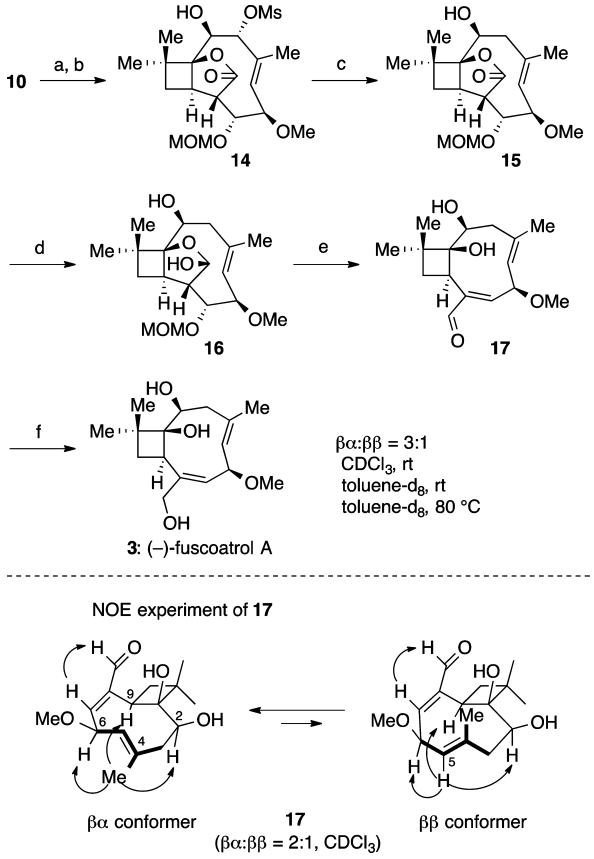
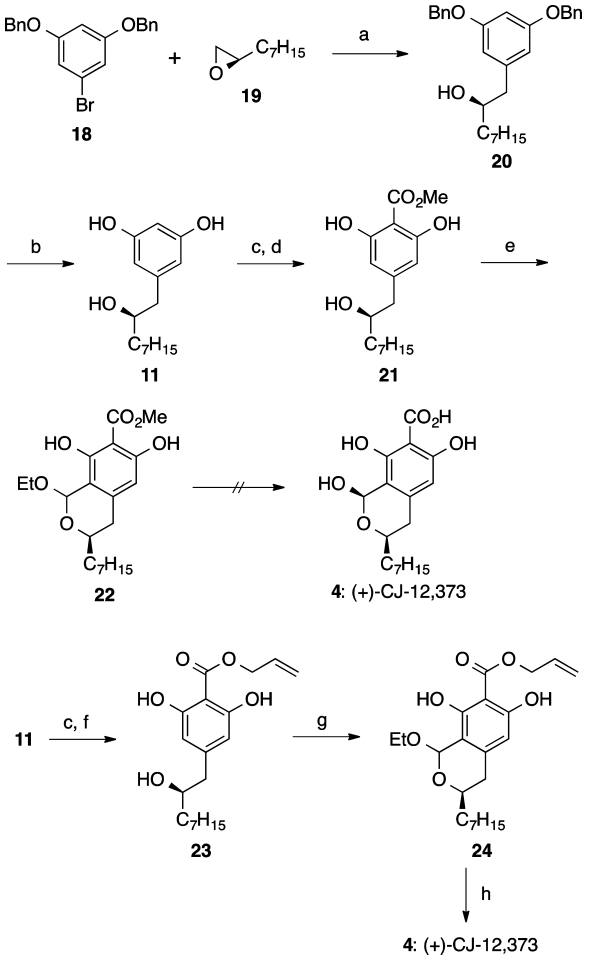
Scheme 2. Synthesis of (−)-Fuscoatrol A (3)[a]



[a]Reagents and conditions: (a) Ms_2O, DMAP, pyridine, quant.; (b) DDQ, CH_2Cl_2, phosphate buffer, quant.; (c) $Pd_2(dba)_3$, *n*-Bu_3P, $NaBH_4$, 1,4-dioxane, 71%; (d) DIBALH, THF, 0 °C, 74% (for two cycles); (e) DBU, THF, 0 °C, 66% (for two cycles); (f) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C, 88%.

Scheme 3. Synthesis of (+)-CJ-12,373 (4)[a]



[a]Reagents and conditions: (a) **18**, *n*-BuLi, then **19**, $BF_3{\cdot}OEt_2$, THF, −78 °C, 95%; (b) H_2, Pd on C, AcOH, MeOH, 93%; (c) CO_2, $KHCO_3$, glycerol, 150 °C, then 1 M aq. HCl; (d) MeI, $NaHCO_3$, DMF, 61% and recovered **11** (39%); (e) $(EtO)_3CH$, TFA, quant.; (f) allyl bromide, $NaHCO_3$, DMF, 51% and recovered **11** (35%); (g) $(EtO)_3CH$, TFA, quant.; (h) $Pd(PPh_3)_4$, pyrrolidine, THF, then aq. NH_4Cl, quant.

diisobutylaluminum hydride (DIBALH) reduction. Exposure of lactol **16** to an amine base resulted in ring opening of the hemiacetal, followed by *β*-elimination, to form *α,β*-unsaturated aldehyde **17**, of which the newly formed trisubstituted olefin had the desired *E*-configuration. The NMR spectra of **17** indicated that two equilibrating conformers exist in a 2:1 ratio in $CDCl_3$ at room temperature. The major conformer was assigned as the *βα* conformer by NOE analysis.[17] Reduction of **17** under Luche conditions finally afforded (−)-fuscoatrol A (**3**), the properties of which were identical to those reported for natural (−)-fuscoatrol A.[4] As reported by Kuznetsova and co-workers,[4] compound **3** was confirmed to exist in $CDCl_3$ solution as two conformers (*βα:ββ* in a 3:1 ratio). In a toluene-d_8 solution at room temperature or 80 °C, the signal intensity ratio of two conformers showed almost no change.

The synthesis of (+)-CJ-12,373 (**4**) and its derivative **22**, which are the precursors of *o*-quinone methide, is outlined in Scheme 3. We chose 3,5-bis(benzyloxy)bromobenzene (**18**) and (2*R*)-heptyloxirane (**19**) as starting materials. According to a known procedure, aryl derivative **18** was prepared by reaction of commercially available 1-bromo-3,5-difluorobenzene with benzyl alcohol/NaH,[18] and chiral epoxide **19** (>99% *ee*) was prepared from 1-nonene by using the elegant hydrolytic kinetic resolution strategy developed by Jacobsen.[19] Bromide **18** was converted into an aryl lithium intermediate, which was treated with epoxide **19** in the presence of $BF_3{\cdot}OEt_2$ to give coupled product **20**. The benzyl groups in **20** were removed by hydrogenolysis to provide resorcinol derivative **11**. A Kolbe−Schmitt reaction of **11** provided the carboxylic acid, which was esterified to methyl ester **21**. Compound **21** was cyclized by an oxa-Pictet−Spengler reaction with triethyl orthoformate[20] to construct a six-membered ring. Product **22** is equivalent to a protected (+)-CJ-12,373 and was expected to be suitable as a precursor for *o*-quinone methide. Conversion of **22** to (+)-CJ-12,373 (**4**) by hydrolysis was examined next. However, despite extensive efforts, deprotected **4** could not be obtained, probably due to its instability to acidic and basic conditions. To circumvent this problem, the carboxylic acid derived from **11** was protected as allyl ester **23**. An oxa-Pictet−Spengler reaction of **23** provided **24**, which was subject to palladium(0)-catalyzed deprotection of the allyl ester, producing (+)-CJ-12,373 (**4**) in an excellent yield after workup with aqueous ammonium chloride. The physical data and spectra for synthetic **4** matched those reported for the natural substance.[5]

With (−)-fuscoatrol A (**3**) and the precursors of *o*-quinone methide (**22** and **4**) in hand, we investigated joining the two fragments and forming the cytosporolide skeleton. To explore

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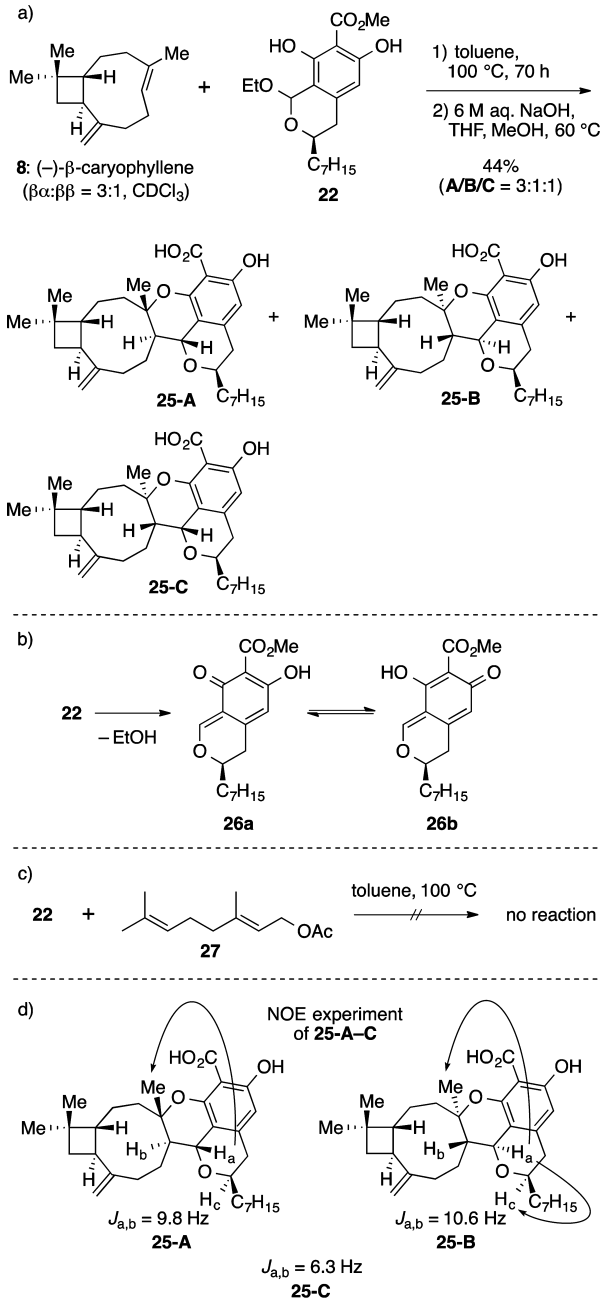
the feasibility of the hetero-Diels–Alder reaction inspired by the proposed biosynthesis of cytosporolide A, we tested the reaction by using commercially available (−)-*β*-caryophyllene (**8**) as a model compound for valuable **3** (Scheme 4a). Various

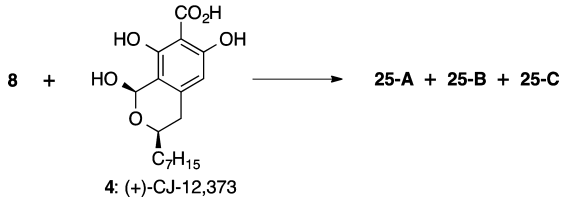
Scheme 4. Hetero-Diels–Alder Reaction of (−)-*β*-Caryophyllene (8) with 22



methods for *o*-quinone methide generation have been reported.[15] Because preliminary experiments had indicated that compound **3** was sensitive to acids, similar to (+)-pestalotiopsin A (**12**), we generated the *o*-quinone methide intermediate under thermal conditions without activation by Lewis or protonic acids.[21] A solution of **8** (1.5 equiv) and **22** (1 equiv) in toluene was heated at 100 °C, which induced elimination of EtOH from **22**, presumably generating an equilibrating mixture of *o*-quinone methide **26a** and *p*-quinone methide **26b** (Scheme 4b). The former intermediate underwent a hetero-Diels–Alder reaction with dienophile **8** to produce cycloadducts as a mixture of methyl and ethyl esters. After alkaline hydrolysis, a diastereomeric mixture of pentacyclic acids **25-A**, **25-B**, and **25-C** was obtained in a 3:1:1 ratio. Notably, complete chemoselectivity and regioselectivity were achieved in the hetero-Diels–Alder reaction. Although caryophyllene **8** possesses two double bonds that can serve as a dienophile, the endocyclic *trans*-double bond is expected to be more reactive because of the high strain of the (*E*)-cyclononene ring. Indeed, the *o*-quinone methide intermediate exclusively reacted with the endocyclic double bond in **8**, whereas treatment of **22** with geranyl acetate (**27**) under the same conditions resulted in no reaction (Scheme 4c). Regarding the regioselectivity, the C–O bond was formed at the more substituted alkene site, similar to previous examples,[15] and the regioisomeric cycloadduct was not detected. NOE experiments for **25-A** to **-C** showed that the major isomer **25-A** had the correct stereochemistry for (+)-cytosporolide A (**2**) (Scheme 4d),[22] indicating that the hetero-Diels–Alder reaction preferentially proceeded between the minor *ββ* conformer of **8** and *o*-quinone methide **26a**. The diastereomeric ratio of cycloadducts remained unchanged upon extending or shortening the reaction time, suggesting the irreversibility of the cycloaddition.

To follow the biosynthesis more closely, (+)-CJ-12,373 (**4**) was used for the hetero-Diels–Alder reaction (Table 1). The

Table 1. Hetero-Diels–Alder Reaction of (−)-*β*-Caryophyllene (8) with 4



entry	conditions	yield (A/B/C)[a]
1	toluene, 100 °C, 7 h	44% (7:1:1)
2	toluene, 150 °C, 3 h	14% (4:1:0)
3	silica gel (500 wt%), toluene, 100 °C, 15 h	32% (5:3:0)
4	silica gel (500 wt%), toluene, 150 °C, 2 h	21% (12:6:1)

[a]Ratio determined by ^{1}H NMR analysis.

reaction of **8** with **4** proceeded smoothly at 100 °C to provide the cycloadducts **25-A–C** in a ratio of 7:1:1 (entry 1). In this case, the reactivity of **4** was higher than **22** and the diastereoselectivity was improved. However, raising the reaction temperature to 150 °C reduced the yield of cycloadducts considerably (entry 2). Addition of silica gel, a mild accelerator of Diels–Alder reactions,[23] resulted in a decrease in the diastereoselectivity (entries 3 and 4). These results (Scheme 4 and Table 1) suggest that both **22** and **4** can generate *o*-quinone methide intermediates under thermal conditions and react preferentially with the *ββ* conformer of caryophyllene **8**, despite it being the minor conformer, to afford a cycloadduct with the desired stereochemistry.[24]

The stereochemical outcomes obtained in the reaction of **8** with **4** or **22** can be explained by the transition states depicted in Figure 3. The more stable *βα* conformer of **8** is approached by the *Re*-face of *o*-quinone methide **5** (or **26a**) inducing the steric repulsion by the exocyclic olefinic methyl group in **8** (**TSC**), whereas the approach of the *Si*-face is interrupted by the steric hindrance of the alkyl side chain (C_7H_{15} group) in **5**

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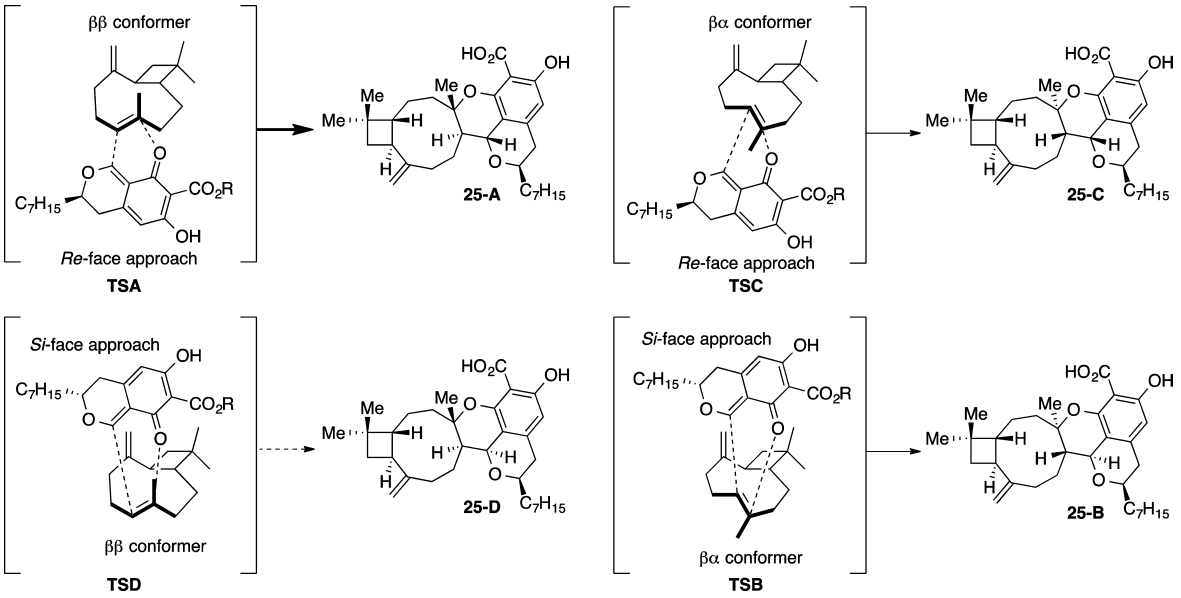
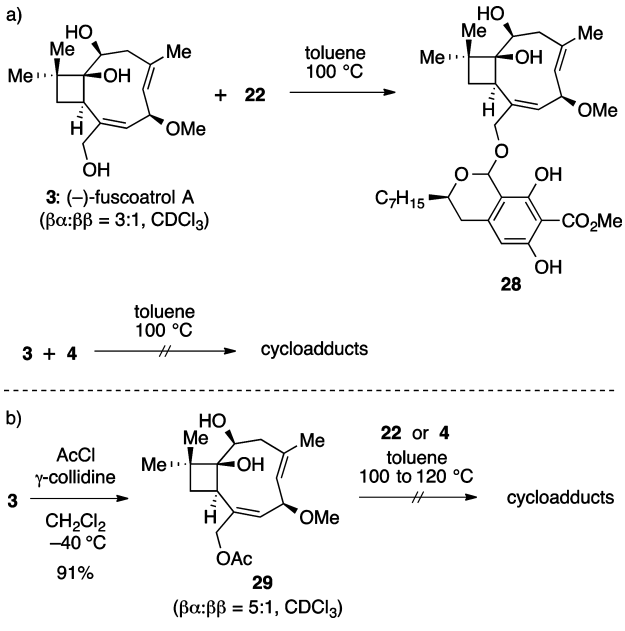


Figure 3. Plausible transition states for the hetero-Diels–Alder reaction of (−)-*β*-caryophyllene (**8**) with **22** or **4**.

(or **26a**) (**TSB**). Therefore, to avoid these repulsions, the cycloaddition proceeds predominantly through transition state **TSA**, leading to **25-A**. The energy difference of the two conformers (*βα* and *ββ*) of **8** is calculated to be 0.75 kcal/mol,[8] which is presumably smaller than the steric repulsions encountered in transition states **TSB** and **TSC**. In contrast, (−)-guajadial (**6**) and (+)-psidial A (**7**) are formed by the cycloaddition involving the *βα* conformer of **8**.[9] In the reaction of **8** with **4** or **22**, the configuration of the C_7H_{15} group in the *o*-quinone methide intermediate plays a significant role in controlling the stereoselectivity.

Encouraged by our model studies, we conducted the hetero-Diels–Alder reaction using (−)-fuscoatrol A (**3**) as a dienophile. Heating a mixture of **3** and **22** in toluene at 100 °C resulted in the formation of a new product (Scheme 5a). However, it was 1,4-adduct **28** (~20% yield) generated from 1,4-addition of the primary hydroxy group in **3** to the *o*-quinone methide intermediate. The mixture containing **28** was heated further, but the expected elimination followed by cycloaddition did not occur and only slow decomposition was observed. In addition, the reaction of **3** with **4** gave no cycloadduct. These results demonstrated that protection of the primary hydroxy group in **3** would be required for the desired hetero-Diels–Alder reaction. Therefore, selective acetylation of **3** by AcCl and *γ*-collidine afforded monoacetate **29**,[25] the conformational ratio (*βα*:*ββ*) of which was 5:1 (Scheme 5b). Contrary to our expectations, the reaction of **29** with **22** or **4** produced only unidentified compounds and no cycloadduct was formed. Similar observations were made when the primary hydroxy group was protected as a silyl ether.

Scheme 5. Unsuccessful Hetero-Diels–Alder Reactions



Next, we turned our attention to diacetate **30** (Scheme 6). Thorough acetylation of **3** gave diacetate **30**, which occurs as a 5:3 mixture of *βα* and *ββ* conformers. Gratifyingly, the reaction of **30** (1 equiv) with **22** (1.5 equiv) proceeded under thermal conditions, giving cycloadduct **31** as a single diastereomer. Based on the NOE experiments, the stereochemistry of **31** corresponded to (+)-cytosporolide A (**2**).[26] Product **31** was obtained as an acid, because it was suspected that the initially formed cycloadduct may undergo hydrolysis with even traces of water in the solvent. The remaining task was to remove two acetyl groups from **31**. However, all attempts to deprotect **31** to (+)-cytosporolide A (**2**) failed because of the stability of the acetyl group at C6–OH and the lability of **2** under basic conditions, which necessitated changing the protecting group.

Eventually, we accomplished a total synthesis of (+)-cytosporolide A (**2**) as shown in Scheme 7. The primary hydroxy group in **3** was selectively protected by a benzoyl (Bz) group and the resultant secondary alcohol was protected by a triethylsilyl (TES) group to yield diprotected **32** as a conformational mixture of 3:1 (*βα* and *ββ*). In agreement with **30**, the hetero-Diels–Alder reaction of **32** with **22** was highly diastereoselective for the formation of the three newly formed stereogenic centers, which were confirmed to be the correct configuration by NOE analysis.[27] Cycloadduct **33** was also obtained from the reaction with **4**.[28] In both cases, the isolable product was only diastereomer **33**, indicating that the

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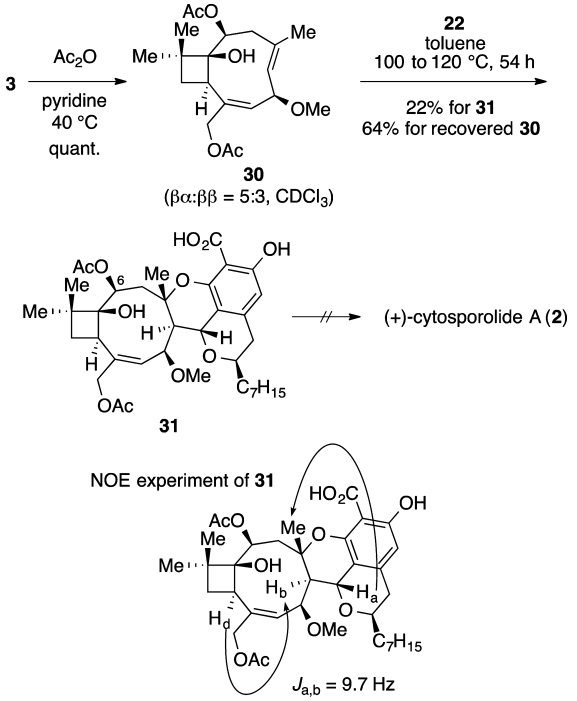
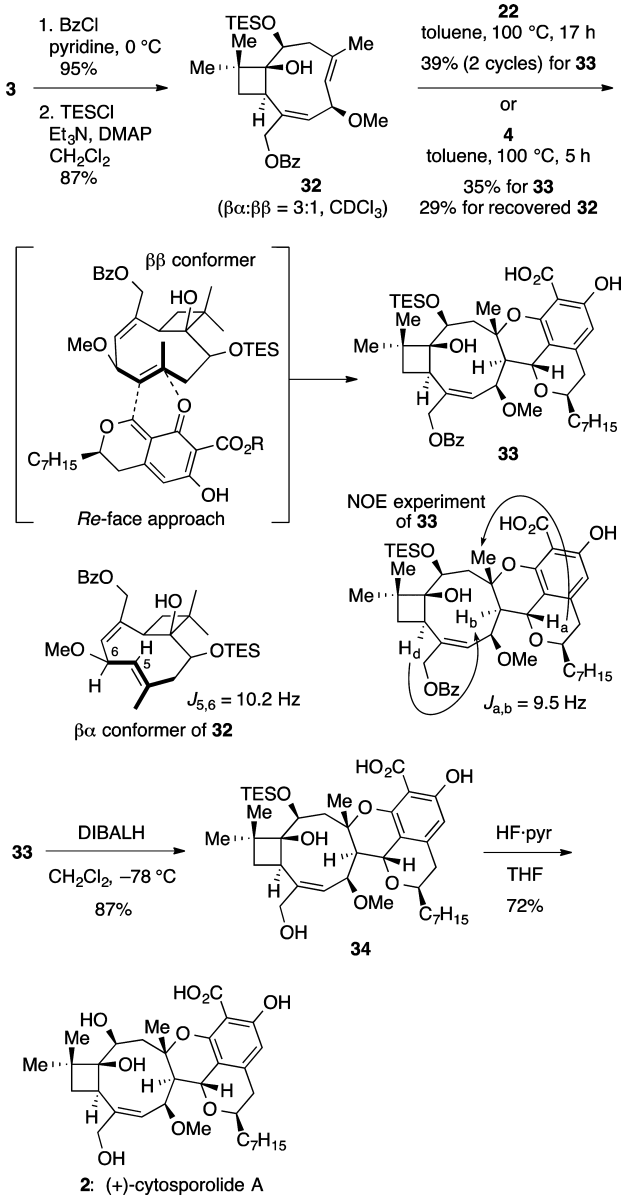
Scheme 6. Hetero-Diels–Alder Reaction of Diacetate 30 with 22



Scheme 7. Completion of the Total Synthesis of (+)-Cytosporolide A (2)



reaction proceeded exclusively through the transition state corresponding to **TSA** in Figure 3. The presence of the methoxy group at C-6 in **32** probably prevented the approach of the *o*-quinone methide intermediate in the transition states involving the *βα* conformer, in which the methoxy group adopts a pseudoequatorial orientation and may contribute to the steric repulsion. Although the yield is still moderate, the biomimetic hetero-Diels–Alder reaction provides concise access to the pentacyclic ring system of the cytosporolide skeleton. During the last deprotection, the order of events was important. First, the Bz group was reductively removed from **33** by DIBALH followed by a workup with diluted hydrochloric acid to afford alcohol **34**. Finally, treatment of **34** with HF·pyridine allowed clean deprotection of the TES group, and the resulting mixture was purified by reverse-phase chromatography to provide (+)-cytosporolide A (**2**). The properties of the synthetic sample were identical in all respects to those reported for natural cytosporolide A.[1] This fact verified that natural cytosporolide A has the structure suggested by Spence and George.[2]

CONCLUSION

In summary, we have completed the first total synthesis of (+)-cytosporolide A (**2**) by using the putative biosynthetic hetero-Diels–Alder reaction between (−)-fuscoatrol A (**3**) and the *o*-quinone methide generated from (+)-CJ-12,373 (**4**). To achieve this goal, we synthesized (−)-fuscoatrol A (**3**) from synthetic intermediate **10** in our previous total synthesis of (+)-pestalotiopsin A (**12**). Furthermore, (+)-CJ-12,373 (**4**) was synthesized through a Kolbe–Schmitt reaction and an oxa-Pictet–Spengler reaction starting with aryl derivative **18** and chiral epoxide **19**. The hetero-Diels–Alder reaction of diprotected **32** and *o*-quinone methide precursor **4** or **22** showed complete chemo-, regio-, and stereoselectivity to produce cycloadduct **33** as a single isomer. In this reaction, protection of the two hydroxy groups in fuscoatrol A was required. Instead of this, enzymes or other substances may contribute to the assembly of the cytosporolide skeleton in nature. Our total synthesis has validated the biogenetic hypothesis and established the structure of cytosporolide A.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b11438.

Experimental procedures and characterization data for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(28) In this case, 29% of the starting material **32** was recovered. The ratio of two conformers of the recovered **32** was confirmed to be unchanged ($\beta\alpha$:$\beta\beta$ = 3:1, $CDCl_3$).

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