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Asymmetric Total Synthesis of Danicalipin A and Evaluation of Biological Activity

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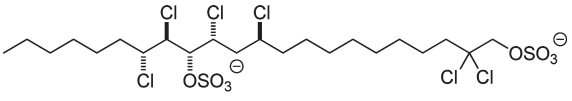
ABSTRACT



Danicalipin A

Asymmetric total synthesis of danicalipin A was achieved. The synthesis was characterized by diastereoselective introduction of chlorine atoms. Biological activities with synthetic danicalipin A, its enantiomer, and racemate were also evaluated toward brine shrimp. Both enantiomers of danicalipin A showed almost the same activity.

Danicalipin A (**1**), a major component of a group of chlorosulfolipids (CSLs) obtained from *Ochromonas danica*, was first detected more than four decades ago along with its congeners, representing a new class of lipids with one to six chlorine atoms (Figure 1).[1] Although the planar structure of **1** was reported in 1973,[1a] the absolute and relative stereochemistries of **1** were not determined until 2009. In that year, Vanderwal reported the determination of the relative stereochemistries of **1** based on a total synthesis of a racemic mixture and of the absolute configuration using naturally derived material.[2] Meanwhile, Okino described an absolute structural determination of **1** from a natural sample obtained from cultured *O. danica* using a combination of *J*-based configuration analysis (JBCA) and the modified Mosher's method.[3] These two reports reached the same conclusion: (11*S*, 13*R*, 14*S*, 15*S*, 16*R*), as shown in Figure 1. It has been reported that **1** shows a wide range of biological activities, such as toxicity

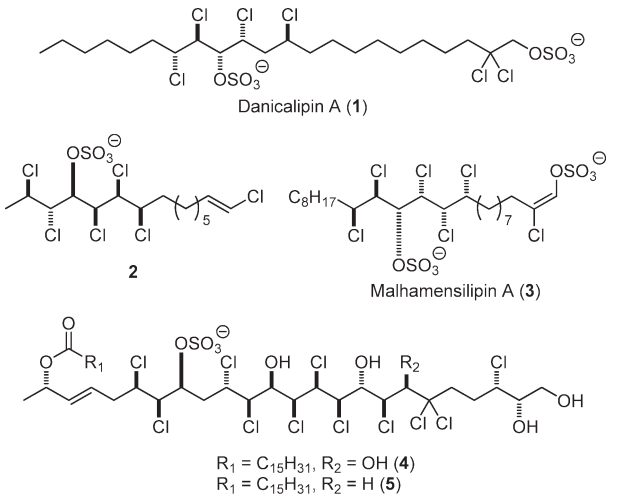


Figure 1. Chlorosulfolipids (CSLs).

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(3) Kawahara, T.; Kumaki, Y.; Kamada, T.; Ishii, T.; Okino, T. *J. Org. Chem.* **2009**, *74*, 6016–6024.

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to fish[4] and invertebrates,[5] inhibition of bacterial growth, and lysis of mammalian erythrocytes.[6] However, the biological mechanism of **1** has not been revealed due to the difficulty of obtaining molecular probes derived from natural sources. Therefore, elucidation of the mechanism using an artificially synthesized molecular probe is highly desirable. Due to the unprecedented structure and interesting biological activity of **1**, along with other CSLs (**2**−**5**),[7] these compounds have been the subject of much attention from synthetic chemists.[8] In 2009, Carreira accomplished an elegant first total synthesis of chlorosulfolipid **2**.[9] After this, total syntheses of **1**−**3** have been achieved by two groups: racemic **1** and optically pure **3** by Vanderwal[2,10] and optically active **2** by Yoshimitsu and Tanaka.[11] In this report, we describe an asymmetric total synthesis of danicalipin A (**1**) and explore the biological activity of **1** and its enantiomer toward brine shrimp.

For the total synthesis of **1**, although a ring-opening reaction of a *cis*-epoxide by a chloride would be expected to be effective for the construction of *syn*-chlorohydrin at C13 and C14, Carreira first reported that epoxide ring-opening using substrates containing intramolecular chlorides resulted in an undesired product of diastereomers due to the generation of intramolecular chloronium ions (Figure 2, eq 1).[9a] Vanderwal also observed the similar phenomenon.[2] To avoid this side reaction, an epoxide ring-opening reaction using a substrate without chloride may be employed at an early stage of the total synthesis. Meanwhile, to synthesize the dichloride at C15 and C16 with *anti* configuration, *anti*-addition of a molecular chlorine equivalent to an *E*-olefin was considered. However, because a previous study by Yoshimitsu and Tanaka indicated that the addition produced a *syn*-adduct along with the *anti*-adducts,[11a] improvement of the selectivity of the *anti*-addition reaction was concluded to be important

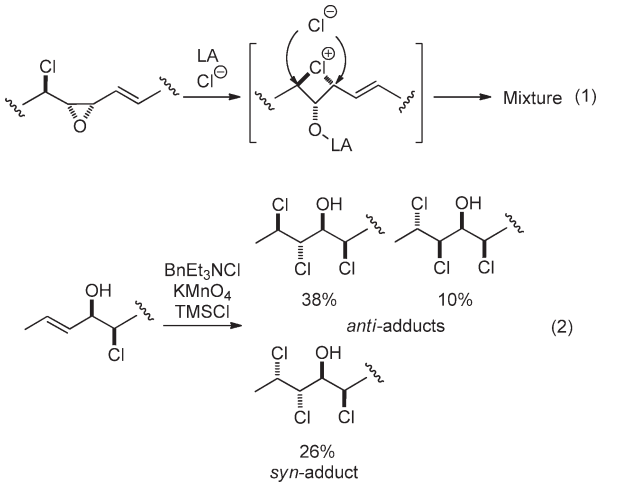
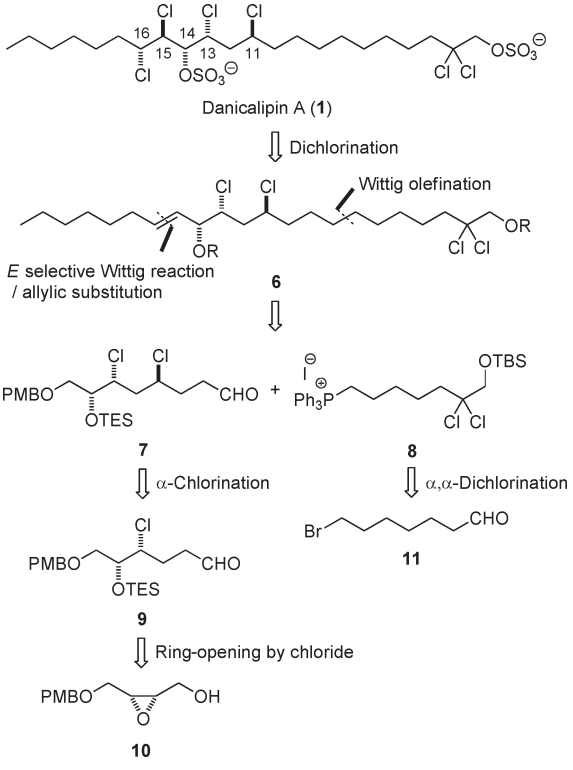


Figure 2. Previous synthetic problems to overcome.

Scheme 1. Retrosynthetic Analysis of Danicalipin A



(Figure 2, eq 2). With these considerations in mind, the following retrosynthetic analysis of **1** was planned (Scheme 1). As mentioned above, **1** was expected to be obtained from *E*-olefin **6** by *anti*-addition of the molecular chlorine equivalent, which would be derived from aldehyde **7** and phosphonium salt **8** by Wittig olefination. We envisioned that aldehyde **7** would be accessed by α-chlorination with aldehyde **9**, as reported by Jørgensen,[12] which would be synthesized from the known *cis*-epoxide **10**.

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(6) (a) Hansen, J. A. *Physiol. Plant.* **1973**, *29*, 234–238. (b) Halevy, S.; Saliternik, R.; Avivi, L. *Int. J. Biochem.* **1971**, *2*, 185–192. (c) Magazanik, A.; Halevy, S. *Experientia* **1973**, *15*, 310–311.

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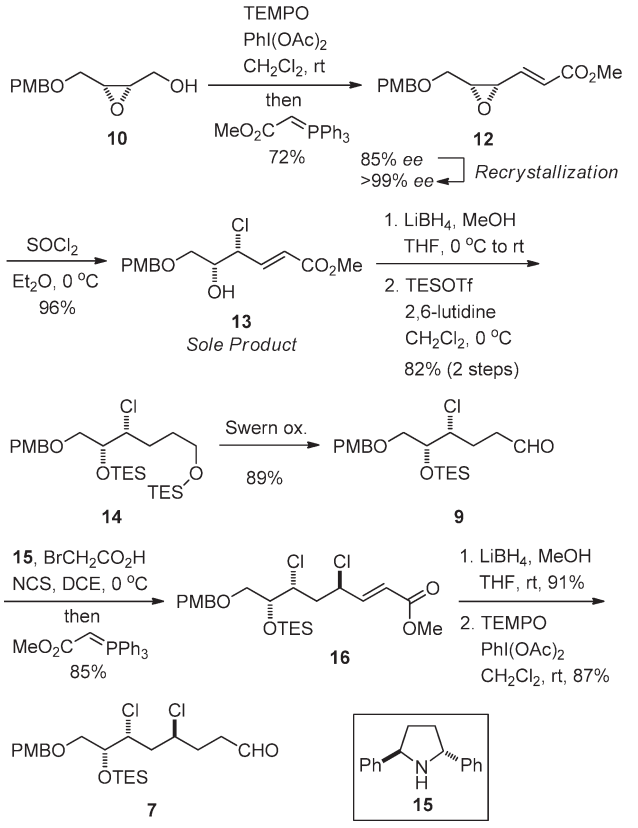
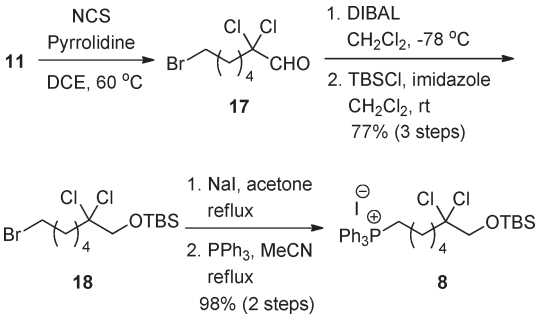
Scheme 2. Synthesis of Aldehyde **7**



The synthesis of **1** commenced with the known chiral epoxide **10**,[13] derived *via* Sharpless–Katsuki asymmetric epoxidation, which was transformed into α,β-unsaturated ester **12** in a one-pot operation (Scheme 2).[14] At this stage, the enantiomeric excess of **12** was enhanced from 85% ee to >99% ee by recrystallization. Regioselective ring-opening of the epoxide by chloride was achieved by treatment with $SOCl_2$ to give chlorohydrin **13** as a sole product in excellent yield.[15] Reduction of the α,β-unsaturated ester with $LiBH_4$ afforded a saturated primary alcohol, the alcohol groups of which were protected as TES ethers. TES ether **14** was then converted to aldehyde **9**, the precursor for α-chlorination, by Swern oxidation. After extensive investigations of catalysts, additives, and temperatures for the diastereoselective α-chlorination reaction, the combination of (*R*,*R*)-2,5-diphenylpyrrolidine (**15**)[12] and bromoacetic acid in 1,2-dichloroethane at 0 °C was found to achieve the best selectivity (diastereoselectivity >20:1; ratio of mono- to dichlorination >20:1), furnishing a labile α-chloroaldehyde which was isolated as α,β-unsaturated ester **16** by adding a Wittig reagent in a one-pot operation. The α,β-unsaturated ester

Scheme 3. Synthesis of Phosphonium Salt **8**



16 was converted to **7** by $LiBH_4$ reduction of the α,β-unsaturated ester to a saturated alcohol, followed by TEMPO oxidation.

With **7** in hand, we next turned our attention to the synthesis of another coupling partner, the phosphonium salt **8**. Clean conversion of the known aldehyde **11**[16] to α,α-dichloroaldehyde **17** was accomplished by treatment with NCS in the presence of a catalytic amount of pyrrolidine at 60 °C without the formation of byproduct such as α-monochloroaldehyde or aldol products (Scheme 3). DIBAL reduction and subsequent protection of the resultant alcohol gave TBS ether **18**, which was converted into **8** in two steps.

Wittig reaction between **7** and **8** furnished olefin in 83% yield (Scheme 4). Hydrogenation and deprotection of the PMB group using Pearlman's catalyst gave alcohol **19**, which was transformed to α,β-unsaturated ester **20** with *E* geometry as a major product by treatment with a Wittig reagent[17] derived from $(n\text{-Bu})_3P$ after generation of aldehyde. To convert the ester into an *n*-hexyl group, **20** was converted into acetate **21** and allylic substitution[18] of **21** with $n\text{-}C_5H_{11}MgBr$ and Li_2CuCl_4 afforded olefin **22**. Because dichlorination with **22** resulted in a complex mixture, the silyl ethers were removed. After numerous investigations of the *anti*-selective dichlorination reaction under Markó's conditions,[19] we found that an *anti*-addition reaction of a molecular chlorine equivalent took place with high *anti* selectivity when the reaction was carried out in octane at 90 °C. The desired hexachloride **23** was obtained in 39% yield along with another *anti*-adduct (28%) as a minor diastereomer after purification by HPLC. All spectroscopic data of **23** were completely identical with those of an intermediate synthesized by Vanderwal. According to Vanderwal's synthesis, two sulfate groups were introduced into two hydroxyl groups in **23** by treatment with $ClSO_3H$, achieving the asymmetric total synthesis of danicalipin A (**1**). The $[\alpha]_D$ value of synthetic **1** was

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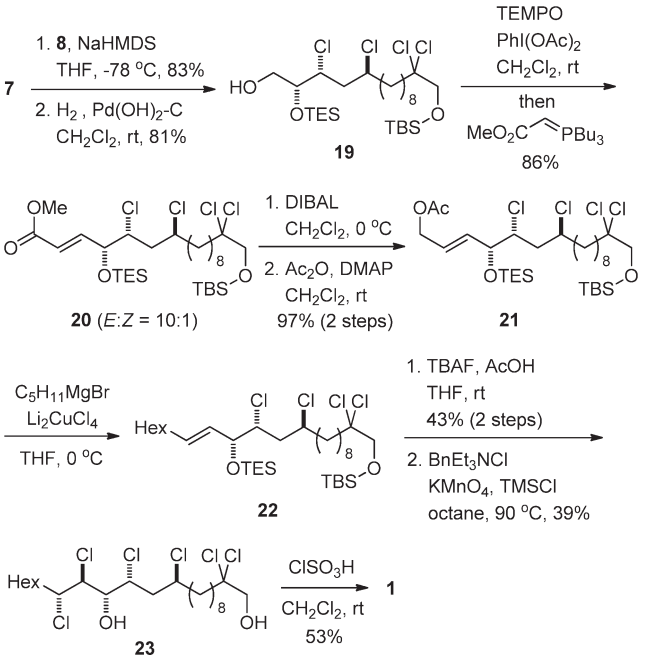
Scheme 4. Total Synthesis of Danicalipin A



+31.5 (c = 0.25, MeOH), which corresponded to the reported $[\alpha]_D$ value [+38 (c = 0.78)].[2]

Next, we investigated the toxicities of **1**, *ent*-**1**—which was synthesized *via* the same scheme from *ent*-**10**—and *rac*-**1**. Table 1 shows the LC_{50} values (50% lethal concentration) for brine shrimp. The biological activity of synthetic **1** (LC_{50} = 2.1 μg/mL) was identical to that of a natural sample. The enantiomer *ent*-**1** and the *rac*-**1** showed almost the same activity (LC_{50} = 2.4 μg/mL) as natural **1**. These results clearly indicate that the absolute configuration of danicalipin A has no effect on its toxicity in brine shrimp.

Table 1. Toxicity of Synthetic **1**, *ent*-**1**, and *rac*-**1** in Brine Shrimp

entry	compound	LC_{50} (μg/mL)[a]
1	Natural **1**[b]	2.2
2	Synthetic **1**	2.1
3	Synthetic *ent*-**1**	2.4
4	Synthetic *rac*-**1**[c]	2.4

[a] LC_{50}: median lethal concentration against brine shrimp. [b] See ref 3. [c] Prepared by mixing **1** and *ent*-**1** in a 1:1 ratio.

In summary, enantioselective total syntheses of both enantiomers of danicalipin A were achieved in 17 steps (the longest linear sequence) from the known epoxide **10**. The synthesis featured regioselective construction of *syn*-chlorohydrin *via* epoxide, diastereoselective α-chlorination of aldehyde, and dichlorination of *E*-olefin. LC_{50} evaluation of **1** and *ent*-**1** revealed that the stereochemistry of **1** did not affect its activity. Syntheses of analogues of **1** using a modified Grignard reagent in allylic substitution reactions and biological testing of the products are currently underway.

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Supporting Information Available. Experimantal procedure and copies of ^{1}H NMR and ^{13}C NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.