Syntheses and Biological Activities of Danicalipin A Derivatives

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Abstract: Synthesis of three derivatives of danicalipin A, tetrachloride, trisulfate and fluorescent probe was achieved through Wittig reaction strategy. Toxicity of the derivatives against brine shrimp was also investigated to provide useful information for the biological activity; i) less chloride derivative showed the similar toxicity to danicalipin A, ii) the amphiphilic property, a characteristic feature of danicalipin A, was crucial because trisulfate considerably decrease the toxicity and iii) fluorescent derivative kept brine shrimp toxicity of danicalipin A.

Keywords: Chlorosulfolipids • Fluorescent probe • Structure-activity relationship

Introduction

Danicalipin A, a member of chlorosulfolipids (CSLs)[1-4] representing danicalipin A (**1**)[5-13], malhamensilipin A (**2**),[14-16] and mytilipin A-C (**3**-**5**),[17-26] was first discovered from *Ochromonas danica* along with its congeners with one to six chlorine atoms. Although the planar structure of **1** was reported in 1973,[5-9] the relative and absolute configurations of **1** were assigned as shown in Figure 1 in 2009 by Vanderwal[27] and Okino.[28] It has been reported that CSLs show a wide range of biological activities such as toxicity to fish[29, 30] and

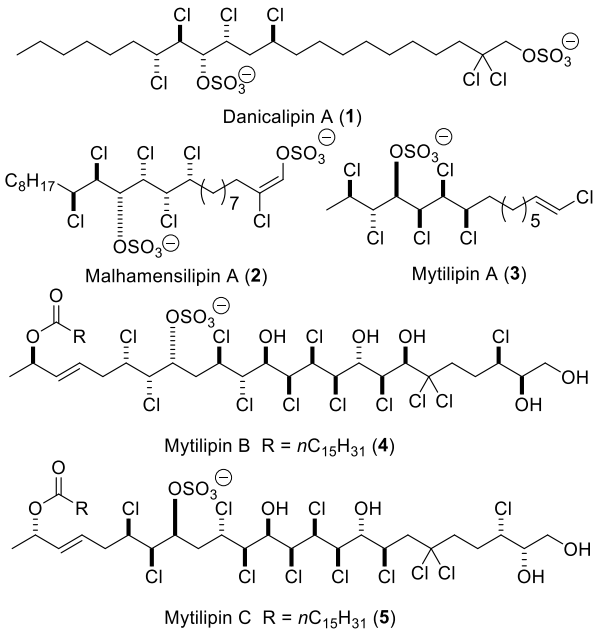
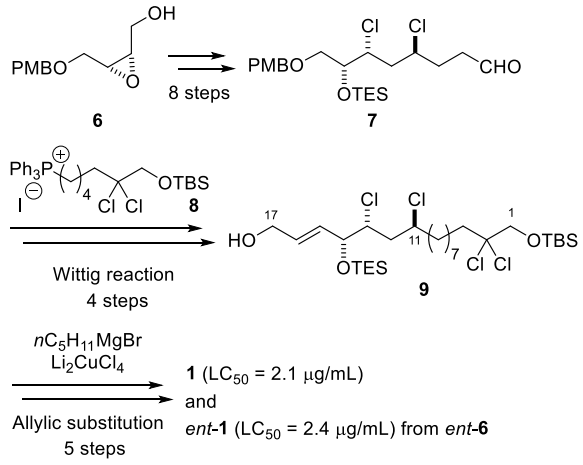


Figure 1. Chlorosulfolipids.

invertebrates,[31, 32] inhibition of bacterial growth, and lysis of mammalian erythrocytes.[33-35] Concerning to structure-activity relationship of **1**, Carreira has described a relationship between biological activities and configuration of **1**[36] by preparing some diastereomers of **1**. Toward direct elucidation of the molecular mechanism with **1** against the biological activities, a molecular probe provided through artificial synthesis is essential because the natural sample of **1** is difficult to be transformed into the desired probe.

Prior to the synthesis of the derivatives of **1**, we have achieved convergent total synthesis of (+)-**1** from optically active epoxide **6** via Wittig reaction (C1-7 installation) followed by allylic substitution reaction (C18-22) with Grignard reagent and copper catalyst[37] to reveal that both enantiomers of **1** show the similar toxicity against brine shrimp, indicating small effect of the absolute configurations of **1** toward the biological activity (Scheme 1).[38-40] In order to obtain



Scheme 1. Previous total synthesis of **1**.

more information about the biological activities, the preparations of three derivatives **10**-**12** were next planed by modification of reaction conditions at the late stage of the total synthesis (Figure 2). Tetrachloride derivative **10**, lack of chlorides at C15 and C16, is expected to have similar biological

activities to **1** because Okino has demonstrated that congeners of **1**, such as **A** and **B**, containing less chlorides maintained the biological activities.[28] Trisulfate derivative **11**, assumed to be a hydrophilic compound, is thought to be an appropriate compound to confirm the amphiphilic property, a characteristic feature of **1**, is essential for the biological activities. Dansyl derivative **12** has a fluorescent function which is expected to be a chemical tool for confirming a localization of **1** in brine shrimp if **12** shows brine shrimp toxicity. In the present paper, the synthesis of the derivatives **10**-**12** and evaluation of their toxicity against brine shrimp are reported.

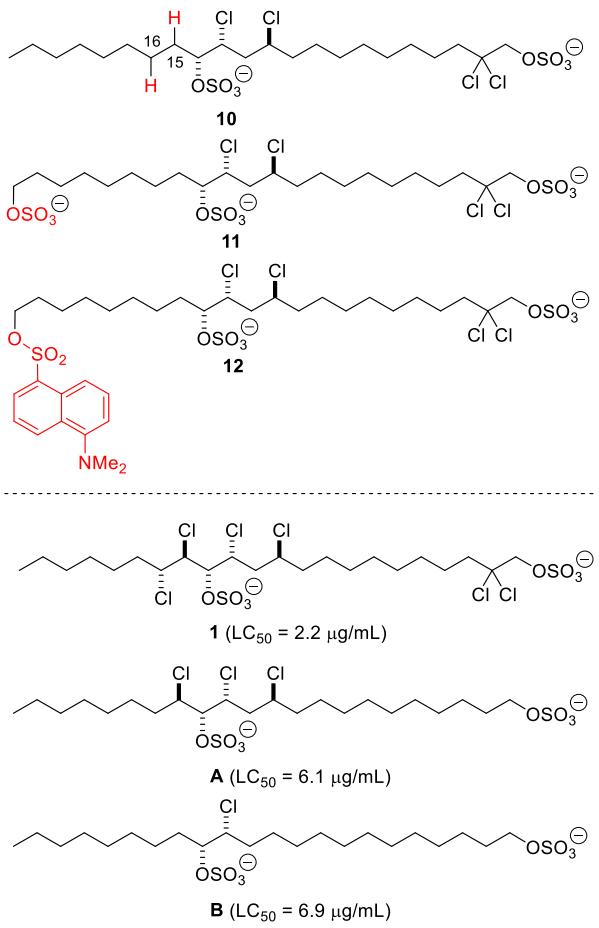
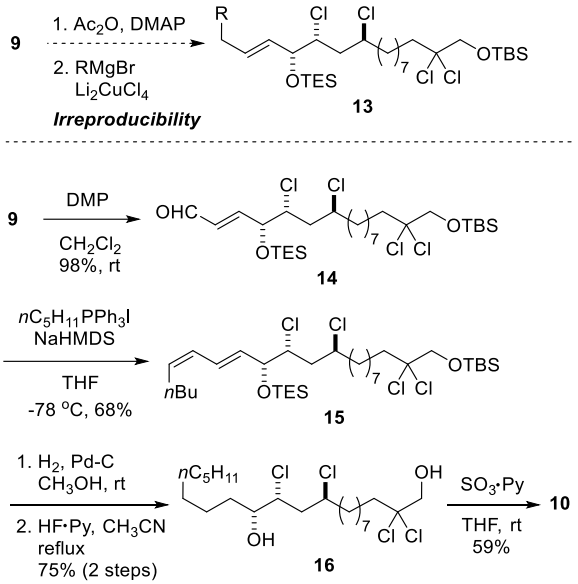


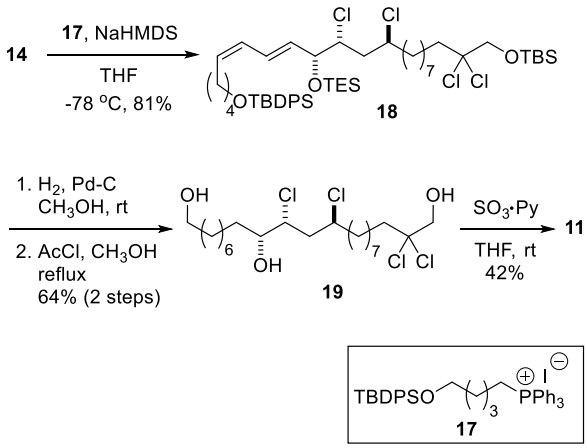
Figure 2. Derivatives **10**-**12**

Results and Discussion

The synthesis of derivative **10** was first attempted by the allylic substitution reaction with Grignard reagent and copper catalyst according to our total synthesis of **1**. Although commercially available Grignard reagent $nC_5H_{11}MgBr$ for **1** or others such as C_2H_5MgBr proceeded to give the corresponding target compound, Grignard reagents prepared from the corresponding alkyl halide for the preparation of **11** and **12** showed irreproducibility in the allylic substitution reaction after extensive optimizations (Scheme 2, upper). Although a reason of this irreproducibility is uncertain, we presume that a generation of the Grignard reagent was not enough to proceed the allylic substitution reaction. Thus, we envisioned Wittig reaction as the homologation strategy (Scheme 2, lower). Alcohol **9** was oxidized into an aldehyde **14** which was subjected to the Wittig reaction conditions, giving diene **15** in good yield (68%). Hydrogenation of diene functionality followed by deprotection of silyl groups provided diol **16**. Treatment of SO_3-Py with **16** furnished the tetrachloride derivative **10**.



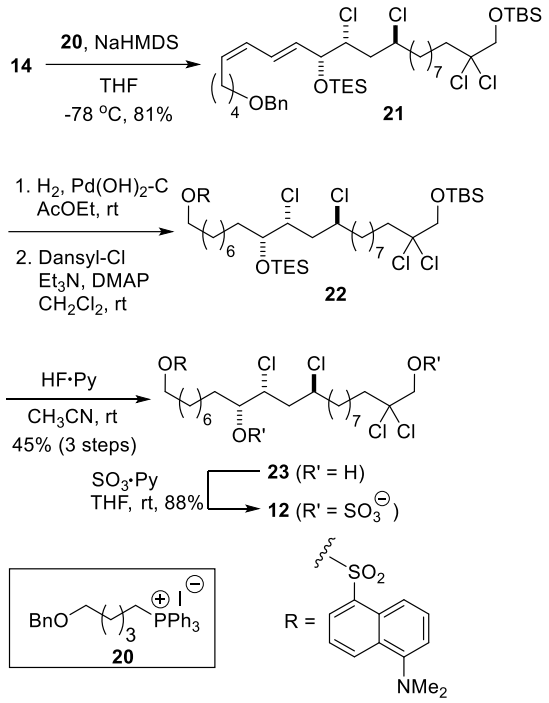
Scheme 2. Synthesis of tetrachloride derivative **10**.



Scheme 3. Synthesis of trisulfate derivative **11**.

Synthesis of trisulfate derivative **11** was started from Wittig reaction with functionalized phosphonium salt **17**, giving diene **18** (Scheme 3). Similar procedures for further conversion via triol **19** were also successful to afford **11** in 42% yield.

Dansyl derivative **12** was prepared with phosphonium salt **20** including benzyl ether for the selective introduction of the fluorescent function into C22. Wittig reaction proceeded to furnish diene **21** in 81% yield. Treatment of H_2 in the presence of $Pd(OH)_2$-C, providing a mono alcohol, followed by sulfonylation reaction gave sulfonate **22**. The final sulfate formation was performed by the same conditions mentioned above.

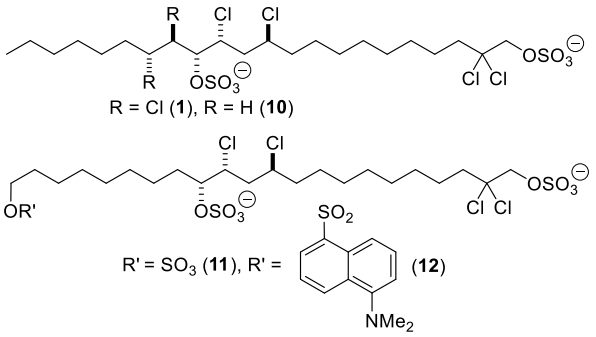


Scheme 4. Synthesis of dansyl derivative **12**.

With synthetic derivatives **10**-**12** in hand, the toxicity assay with brine shrimp (*Artemia salina*) was evaluated as LC_{50} value (50% lethal concentration) as shown in Table 1. As we expected, the toxicity of **10** (LC_{50} = 3.0 μg/mL) was similar to that of **1**. From synthetic point of view, the toxicity of **10** is highly advantageous since synthesis of **10** was much more efficient than that of **1**, not demanding stereoselective dichlorination at C15 and C16. Much weaker activity with **11** was observed (LC_{50} = 34 μg/mL), indicating that the amphiphilic property of **1** plays important role in the toxicity. Installation of fluorescent functional group exhibited moderate activity (LC_{50} = 11 μg/mL). Although the toxicity of **12** was weaker than that of **1** and **10**, the activity was assume to be adequate for the fluorescence probe study.

Table 1. Toxicity of synthetic **10**, **11** and **12** against brine shrimp

Compound	LC_{50} (μg/mL)
1	2.2
10	3.0
11	34
12	11



We next investigated the localization of **12** in brine shrimp with fluorescence microscope. As shown in Figure 3, the strong fluorescent was found within living brine shrimp, meaning the compound was incorporated in the body (concentration = 3.0 μg/mL). [41]

Figure 3. Fluorescent image with compound **12**.

Conclusions

In summary, we have shown the synthesis of danicalipin A derivatives by modification of the synthetic route for **1** and toxicity against the brine shrimp with the derivatives. The evaluation of the toxicity assay has revealed that the synthetic derivative with less chloride exhibited the toxicity and that the amphiphilic property of **1** is crucial for the biological activity. The fluorescent probe with moderate toxicity was observed inside of brine shrimp. The results obtained by the trisulfate and the fluorescent probe are first examples toward elucidating the origin of the toxicity of **1**. Further studies such as preparation of probes and the biological activity are currently underway.

Experimental Section

General Methods.

The IR spectra were recorded on a JASCO FTIR-4100 Type A spectrometer (JASCO corporation, Tokyo, Japan) using a NaCl cell. ESI-MS were obtained on a JEOL JMS-700TZ (JEOL Ltd., Tokyo, Japan) or Bruker Daltonics micro TOF-HS focus spectrometer (Bruker Japan Ltd., Yokohama, Japan). Optical rotations were recorded on a HORIBA SEPA-300 polarimeter (HORIBA Ltd., Kyoto, Japan). The ^{1}H NMR and ^{13}C NMR spectra were recorded using a JNM-EX 400 (400 MHz and 100 MHz) spectrometer (JEOL Ltd., Tokyo, Japan). Chemical shifts were reported in ppm relative to $CHCl_3$ in $CDCl_3$ for ^{1}H NMR (δ = 7.26) and ^{13}C NMR (δ = 77.0). Splitting patterns for ^{1}H NMR were

designated as "s, d, t, q, m, dt, dd, and td". These symbols indicate "singlet, doublet, triplet, quartet, multiplet, doublettriplet, doubletdoublet, and tripletdoublet" respectively. All commercially obtained reagents were employed as received. Analytical TLC was carried out using pre-coated silica gel plates (Wako TLC Silicagel 70F_{254}, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Wakogel 60N 63-212 μm was used for column chromatography.

Aldehyde **14**

To a solution of alcohol **9** (18.5 mg, 28.4 μmol) in CH_2Cl_2 (0.60 mL) was added DMP (15.7 mg, 36.9 μmol) at room temperature under Ar atmosphere. The mixture was stirred for 1 h, quenched with saturated NH_4Cl, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc = 99:1) to give aldehyde **14** (18.0 mg, 27.8 μmol, 98%) as a colorless oil: $[\alpha]_D^{18}$ +37.2 (*c* 0.74, $CHCl_3$); IR (neat) 2954, 2929, 2856, 1733, 1698, 1463, 1415, 1378, 1362, 1255, 1118, 1007, 980, 923, 840, 815, 780, 746, 698, 603 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.11 (6H, s), 0.64 (6H, q, *J* = 7.8 Hz), 0.90 (9H, s), 0.97 (9H, t, *J* = 7.8 Hz), 1.22 - 1.41 (12H, m), 1.50 - 1.66 (2H, m), 1.69 - 1.76 (2H, m), 2.15 - 2.19 (2H, m), 3.92 (2H, s), 4.11 - 4.16 (1H, m), 4.32 (1H, ddd, *J* = 11.8, 4.4, 1.5), 4.69 (1H, td, *J* = 4.1, 1.5), 6.41 (1H, ddd, *J* = 15.6, 8.3, 2.0), 7.03 (1H, dd, *J* = 16.8, 3.9), 9.64 (1H, d, *J* = 7.8); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.4, 4.6 (×2), 6.7, 14.1, 18.2, 24.7, 25.7, 26.3, 28.9, 29.2, 29.6, 38.8, 40.6, 43.4, 60.2, 60.7, 72.0, 73.5, 93.4, 133.3, 153.8, 193.0; HRMS (ESI) m/z: $[M+H]^+$; Calcd for $C_{29}H_{57}O_3Cl_4Si_2$ 649.2606; Found 649.2595.

Olefin **15**

To a solution of phosphonium salt (39.8 mg, 86.4 μmol) in THF (0.50 mL) was added NaHMDS (1.06 M in THF, 73.0 μL, 80.6 μmol) in one portion at −78 °C under an Ar atmosphere. After the mixture was stirred for 30 min, a solution of aldehyde **14** (18.7 mg, 28.8 μmol) in THF (0.50 mL) was added via canula. The mixture was stirred for 20 min, warmed to 0 °C, stirred for 10 min, quenched with saturated $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc = 99:1) to give olefin **15** (12.7 mg, 19.6 μmol, 68%) as a colorless oil: $[\alpha]_D^{21}$ –7.9 (*c* 1.15, $CHCl_3$); IR (neat) 2954, 2928, 2875, 2856, 1738, 1463, 1413, 1378, 1362, 1255, 1152, 1118, 1007, 987, 954, 839, 816, 779, 745, 729, 697, 606 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.11 (6H, s), 0.63 (6H, q, *J* = 7.8 Hz), 0.90 (9H, s), 0.96 (3H, t, *J* = 7.8 Hz), 0.97 (9H, t, *J* = 7.8 Hz), 1.25 - 1.43 (14H, m), 1.50 - 1.62 (3H, m), 1.68 - 1.77 (2H, m), 1.87 (1H, ddd, 14.9, 11.2, 2.4), 2.08 - 2.20 (4H, m), 3.92 (2H, s), 4.14 - 4.24 (2H, m), 4.39 (1H, t, *J* = 4.9), 5.48 (1H, q, *J* = 7.3), 5.74 (1H, dd, *J* = 15.1, 5.9), 6.01 (1H, t, *J* = 11.2), 6.57 (1H, dd, *J* = 15.1, 11.2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.3, 4.9 (×2), 6.8, 13.9, 18.3, 22.3, 24.7, 25.7, 26.4, 27.5, 29.0, 29.3, 29.4, 29.7, 31.8 (×2), 38.9, 41.3, 43.5, 60.9, 63.1, 72.1, 75.3, 93.5, 127.6, 127.7, 130.4, 133.2; HRMS (ESI) m/z: $[M+Na]^+$; Calcd for $C_{34}H_{66}O_2Cl_4NaSi_2$ 725.3266; Found 725.3248.

Diol **16**

To a solution of olefin **15** (12.5 mg, 17.8 μmol) in CH_2Cl_2 (0.50 mL) was added Pd-C (20 wt% Pd on carbon, 2.50 mg) at room temperature under Ar atmosphere. The mixture was stirred for 3 h under H_2 atmosphere, filtered through celite pad, and concentrated *in vacuo* to give a crude bissilyl ether, which was employed directly in the next reaction.

To a solution of crude bissilyl ether in MeCN (0.50 mL) were added HF·Py (5.04 μL, 39.2 μmol) at room temperature under Ar atmosphere.The mixture was stirred for 12 h, quenched with saturated $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo.* The residue was purified by silica gel column chromatography (hexane:EtOAc =99:1 then 85:15) to give diol **16** (6.38 mg, 13.4 μmol, 75%) as a colorless oil: $[\alpha]_D^{26}$ +14.6 (*c* 0.65, $CHCl_3$); IR (neat) 3396, 2925, 2855, 1718, 1579, 1539, 1465, 1377, 1260, 1117, 1075, 920, 801, 706, 656 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, *J* = 6.8 Hz), 1.25 - 1.33 (24H, m), 1.55 - 1.66 (2H, m), 1.74 - 1.80 (2H, m), 1.96 (1H, ddd, 15.1, 11.2, 2.0 Hz), 2.19 - 2.30 (3H, m), 3.61 (1H, td, *J* = 9.3, 3.2 Hz), 3.90 (2H, s), 4.18 - 4.24 (1H, m), 4.29 (1H, dt, *J* = 11.2, 2.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 22.7, 24.8, 25.5, 26.3, 28.9, 29.0, 29.2 (×3), 29.5 (×2), 31.8, 35.0, 38.8, 43.5, 44.1, 61.1, 65.9, 72.1, 74.5, 94.7; HRMS (ESI) m/z: $[M+Na]^+$; Calcd for $C_{22}H_{42}O_2Cl_4Na$ 501.1841; Found 501.1831.

Tetrachloride **10**

Diol **16** (7.60 mg, 15.9 μmol) was dissolved in THF (0.70 mL) and SO_3·Py complex (20.2 mg, 127 μmol) was added . After the mixture was stirred for 90 min, SO_3·Py complex (10.1 mg, 63.6 μmol) was added. After another 15 min, saturated $NaHCO_3$ was added and the suspension stirred for 2 h. The mixture was filtered through a silica plug, eluting with CH_2Cl_2:MeOH = 3:1. The residue was concentrated and purified by silica gel column chromatography (MeOH:CH_2Cl_2 = 3:97 then 20:80) to give tetrachloride **10** (6.00 mg, 9.38 μmol, 59%) as a white solid: $[\alpha]_D^{19}$ +12.8 (*c* 0.40, CH_3OH); IR (neat) 3450, 3383, 2925, 2854, 1735, 1581, 1542, 1465, 1436, 1377, 1258, 1122, 1073, 1009, 930, 822, 702, 678, 638 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 0.90 (3H, t, *J* = 6.3 Hz), 1.25 - 1.69 (26H, m), 1.71 - 1.86 (2H, m), 2.12 (1H, ddd, *J* = 15.1, 10.7, 2.4 Hz), 2.19 - 2.27 (3H, m), 4.15 - 4.22 (1H, m), 4.30 (2H, s), 4.44 (1H, dt, *J* = 6.6, 2.4 Hz), 4.56 (1H, td, *J* = 11.2, 2.9 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.4, 23.7, 25.8, 26.4, 27.3, 30.0, 30.1, 30.3 (×2), 30.4, 30.5, 30.6, 31.8, 33.0, 39.9, 43.5, 45.1, 61.7, 62.0, 75.5, 81.7, 91.2; HRMS (ESI) m/z: $[M+2H]^{2+}$; Calcd for $C_{22}H_{40}O_8Cl_4S_2$ 318.0468; Found 318.0465.

Phosphonium Iodide **17**

To a solution of 5-bromo-1-pentanol (1.99 g, 12.0 mmol) in CH_2Cl_2 (30 mL) were added imidazole (814 mg, 12.0 mmol) and TBDPSCl (2.33 mL, 8.98 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 8h, quenched with EtOH and saturated aqueous $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated in *vacuo* to give a crude TBDPS ether, which was employed directly in the next reaction.

To a solution of TBDPS ether in acetone (70 mL) was added NaI (3.12 g, 20.8 mmol) at room temperature under an Ar atmosphere. The mixture was heated at 50 °C, stirred for 3 h, quenched with saturated $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude iodide, which was employed directly in the next reaction.

To a solution of the crude iodide in MeCN (70 mL) was added Ph_3P (2.73 g, 10.4 mmol) at room temperature under an Ar atmosphere. The mixture was heated at 80 °C, stirred for 12 h, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc = 70:30, then MeOH:CH_2Cl_2 = 25:75) to give phosphonium iodide **17** (5.49 g, 7.68 mmol, 64%) as a yellow solid: IR (neat) 3051, 2929, 2858, 1587, 1484, 1471, 1438, 1389, 1239, 1189, 1112, 996, 822, 745, 722, 705, 689, 613 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 0.98 (9H, s), 1.59-1.65 (6H, m), 3.34-3.42 (2H, m), 3.63 (2H, t, *J* = 5.9 Hz), 7.32-7.40 (6H, m), 7.60 (4H, d, *J* = 7.8 Hz), 7.71-7.79 (12H, m), 7.85-7.88 (3H, m); ^{13}C NMR (CD_3OD, 100 MHz) δ −20.0, 22.5, 23.0, 23.3 (d, *J* = 19.8 Hz), 27.4, 28.1 (d, *J* = 65.9 Hz), 32.6, 64.5, 119.5, 120.4, 128.8 (d, *J* = 9.9 Hz), 130.9, 131.5 (d, *J* = 49.4 Hz), 134.8 (d, *J* = 39.5 Hz), 134.9, 136.3 (d, *J* = 13.2 Hz), 136.6; HRMS (ESI) m/z: M^+; Calcd for $C_{39}H_{44}OPSi$ 587.2902; Found 587.2894.

Olefin **18**

To a solution of phosphonium salt **17** (24.5 mg, 34.2 µmol) in THF (1.0 mL) was added NaHMDS (1.06 M in THF, 30.9 µL, 34.2 µmol) in one portion at −78 °C under an Ar atmosphere. After the mixture was stirred for 30 min, a solution of aldehyde **14** (7.42 mg, 11.4 µmol) in THF (1.0 mL) was added via canula. The mixture was stirred for 20 min, warmed to 0 °C, stirred for 10 min, quenched with saturated $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc = 99:1) to give olefin **18** (8.80 mg, 9.20 µmol, 81%) as a colorless oil: $[\alpha]_D^{19}$ +12.8 (*c* 0.40, $CHCl_3$); IR (neat) 3071, 2930, 2857, 1734, 1656, 1589, 1463, 1428, 1389, 1362, 1256, 1112, 992, 938, 839, 779, 741, 702, 613 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.11 (6H, s), 0.61 (6H, q, *J* = 7.8 Hz), 0.90 (9H, s), 0.96 (9H, t, *J* = 8.3 Hz), 1.05 (9H, s), 1.22 - 1.31 (14H, m), 1.46 - 1.60 (3H, m), 1.68 - 1.77 (2H, m), 1.84 - 1.91 (1H, m), 2.06 - 2.11 (2H, m), 2.15 - 2.19 (2H, m), 3.66 (2H, t, *J* = 5.9 Hz), 3.92 (2H, s), 4.14 - 4.22 (2H, m), 4.33 (2H, t, *J* = 5.4 Hz), 5.61 - 5.73 (2H, m), 6.03 (1H, dd, *J* = 15.1, 10.7 Hz), 6.21 (1H, dd, *J* = 15.1, 10.7 Hz), 7.36 - 7.42 (6H, m), 7.66 (4H, d, *J* = 5.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.3, 4.8, 4.9 , 6.8, 18.3, 19.2, 24.7, 25.4, 25.7, 25.8, 26.5, 26.9, 29.0 (×2), 29.3, 29.7, 32.1, 32.3, 38.9, 41.4, 43.5, 61.0, 63.3, 63.7, 72.1, 75.4, 93.5, 127.6, 128.5, 129.4, 129.5 (×2), 129.9, 132.7, 134.1, 135.6 (×2); HRMS (ESI) m/z: $[M+Na]^+$; Calcd for $C_{50}H_{84}O_3Cl_4NaSi_3$ 979.4392; Found 979.4375.

Triol **19**

To a solution of olefin **18** (8.80 mg, 9.20 µmol) in MeOH (1.0 mL) was added $Pd(OH)_2$-C (30 wt% $Pd(OH)_2$ on carbon, 2.64 mg) at room temperature under Ar atmosphere. The mixture was stirred for 3 h under H_2 atmosphere, filtered through celite pad, and concentrated *in vacuo* to give a crude trisilyl ether, which was employed directly in the next reaction.

To a solution of crude trisilyl ether in MeOH (1.0 mL) were added AcCl (32.7 µL, 46.0 µmol) at room temperature under Ar atmosphere.The mixture was then heated to 80 °C. After the mixture was stirred for 12 h, concentrated *in vacuo.* The residue was purified by silica gel column chromatography (hexane:EtOAc =99:1 then 75:25) to give triol **19** (2.90 mg, 5.87 µmol, 64%) as a yellow oil: $[\alpha]_D^{24}$ +9.5 (*c* 0.50, $CHCl_3$); IR (neat) 3365, 2927, 2854, 1716, 1457, 1259, 1074, 920, 757, 705, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.26-1.46 (24H, m), 1.56-1.68 (2H, m), 1.74-1.78 (2H, m), 1.98 (1H, ddd, *J* = 14.9, 11.2, 2.0 Hz), 2.20-2.36 (4H, m), 3.65 (2H, t, *J* = 6.8 Hz), 3.91 (2H, d, *J* = 6.3 Hz), 4.17-4.24 (1H, m), 4.30 (1H, dt, *J* = 10.7, 2.0 Hz) ; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.7, 25.5, 25.7, 26.3, 28.93, 29.17, 29.23, 29.28, 29.30, 29.37, 29.42, 32.7, 34.9, 38.8, 43.5, 44.0, 61.1, 63.0, 65.9, 72.1, 74.4, 94.7; HRMS (ESI) m/z: $[M+Na]^+$; Calcd for $C_{22}H_{42}Cl_4O_3Na$ 517.1783; Found 517.1780.

Trisulfate ester **11**

Triol **19** (4.50 mg, 9.10 µmol) was dissolved in THF (0.50 mL) and $SO_3 \cdot Py$ complex (17.4 mg, 109 µmol) was added . After the mixture was stirred for 90 min, $SO_3 \cdot Py$ complex (11.6 mg, 72.8 µmol) was added. After another 15 min, saturated $NaHCO_3$ was added and the suspension stirred for 2 h. The mixture was filtered through a silica plug, eluting with CH_2Cl_2:MeOH = 3:1. The residue was concentrated and purified by silica gel column chromatography (MeOH:CH_2Cl_2 = 5:95 then 30:70) to give trisulfate ester **11** (2.80 mg, 3.81 µmol, 42%) as a white solid: $[\alpha]_D^{18}$ +11.5 (*c* 0.13, MeOH); IR (neat) 3853, 3734, 3676, 3649, 3567, 2925, 2854, 1717, 1699, 1653, 1558, 1541, 1507, 1457, 1217, 1077 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 1.24-1.50 (24H, m), 1.59-1.64 (2H, m), 1.69-1.79 (2H, m), 2.05-2.23 (5H, m), 3.96 (2H, t, *J* = 6.3 Hz) 4.26 (2H, s), 4.38-4.42 (1H, m), 4.54 (1H, dt, *J* = 10.7, 2.4 Hz) ; ^{13}C NMR (CD_3OD, 100 MHz) δ 25.8, 26.4, 26.8, 27.4, 30.0, 30.1 (×2), 30.4 (×3), 31.6, 39.9, 43.3, 45.1, 56.8, 61.7, 62.0 (×2), 69.3, 75.5, 81.7, 91.3; HRMS (ESI) m/z: $[M+Na]^+$; Calcd for $C_{22}H_{39}O_{12}Cl_4NaS_3$ 377.0138; Found 377.0133.

Phosphonium Iodide **20**

To a solution of (((5-bromopentyl)oxy)methyl)benzene (354 mg, 1.38 mmol) in acetone (14 mL) was added NaI (622 mg, 4.15 mmol) at room temperature under an Ar atmosphere. The mixture was heated at 50 °C, stirred for 3 h, quenched with saturated $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude iodide, which was employed directly in the next reaction.

To a solution of the crude iodide in MeCN (14 mL) was added Ph_3P (518 mg, 1.98 mmol) at room temperature under an Ar atmosphere. The mixture was heated at 80 °C, stirred for 12 h, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc = 70:30, then MeOH:CH_2Cl_2 = 25:75) to give phosphonium iodide **20** (719 mg, 1.27 mmol, 92%) as a yellow oil: IR (neat) 2859, 1585, 1482, 1436, 1111, 994, 744, 722, 689 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 1.59-1.67 (6H, m), 3.37-3.46 (4H, m), 4.43 (2H, s), 7.23-7.28 (5H, m), 7.67-7.80 (12H, m), 7.85-7.89 (3H, m); ^{13}C NMR

(CD_3OD, 100 MHz) δ 22.5, 23.4 (d, *J* = 16.5 Hz), 28.6 (d, *J* = 65.9 Hz), 29.8, 71.0, 73.9, 119.5, 120.4, 128.6, 128.8, 129.4, 131.5 (d, *J* = 49.4 Hz), 134.8 (d, *J* = 39.5 Hz), 136.2 (d, *J* = 9.9 Hz); HRMS (ESI) m/z: M^+; Calcd for $C_{30}H_{32}OP$ 439.2181; Found 439.2185.

Olefin **21**

To a solution of phosphonium salt **20** (163 mg, 296 μmol) in THF (1.5 mL) was added NaHMDS (1.06 M in THF, 259 μL, 296 μmol) in one portion at −78 °C under an Ar atmosphere. After the mixture was stirred for 30 min, a solution of aldehyde **14** (79.6 mg, 148 μmol) in THF (1.0 mL) was added via canula. The mixture was stirred for 20 min, warmed to 0 °C, stirred for 10 min, quenched with saturated $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc = 99:1) to give olefin **21** (66.0 mg, 81.6 μmol, 55%) as a colorless oil: $[\alpha]_D^{23}$ +27.3 (*c* 1.94, $CHCl_3$); IR (neat) 2930, 2856, 1460, 1254, 1113, 838, 779, 732, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.11 (6H, s), 0.62 (6H, q, *J* = 7.8 Hz), 0.90 – 0.99 (18H, m) 1.26 – 1.32 (9H, m), 1.51 – 1.73 (9H, m), 1.84 – 1.91 (1H, m), 2.06 – 2.22 (4H, m), 3.48 (2H, t, *J* = 6.8 Hz), 3.92 (2H, s), 4.12 – 4.22 (1H, m), 4.30 – 4.40 (2H, m), 4.50 (2H, s), 5.44 – 5.51 (1H, m), 5.75 (1H, dt, *J* = 15.1, 5.9 Hz), 6.02 (1H, t, *J* = 10.7 Hz), 6.51 – 6.58 (1H, m), 7.28 – 7.35 (5H, m) 2H; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 4.9 (×2), 6.8 (×2), 18.3, 24.7, 24.8, 25.7, 25.8, 26.0, 26.2, 26.4, 27.8, 29.0, 29.1, 29.3 (×2), 29.4, 38.9, 41.3, 43.5, 60.9, 63.0, 70.2, 72.1, 72.3, 75.3, 93.5, 127.5, 127.6, 127.8, 128.0, 128.3, 130.7, 132.7, 138.6; HRMS (ESI) m/z: $[M–H]^-$; Calcd for $C_{41}H_{71}O_3Cl_4Si_2$ 807.3724; Found 807.3701.

Diol **23**

To a solution of olefin **21** (27.8 mg, 34.3 μmol) in MeOH (0.40 mL) was added $Pd(OH)_2$-C (30 wt% $Pd(OH)_2$ on carbon, 5.50 mg) at room temperature under Ar atmosphere. The mixture was stirred for 1 h under H_2 atmosphere, filtered through celite pad, and concentrated *in vacuo* to give a crude alcohol, which was employed directly in the next reaction.

To a solution of crude alcohol in CH_2Cl_2 (1.0 mL) were added Et_3N (9.60 μL, 68.6 μmol), DMAP (0.800 mg, 6.86 μmol) and dansyl-Cl (12.0 mg, 44.6 μmol) at room temperature under an Ar atmosphere. The mixture was heated at 35 °C, stirred for 16 h, quenched with saturated $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude sulfonate, which was employed directly in the next reaction.

To a solution of crude sulfonate in CH_3CN (1.0 mL) was added HF-Py (50.0 μL) at room temperature under Ar atmosphere.The mixture was then heated at 65 °C, stirred for 4 h, quenched with saturated $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc = 95:5, 90:10, 85:15 then 80:20) to give diol **23** (11.2 mg, 15.4 μmol, 45%) as a colorless oil: $[\alpha]_D^{19.0}$ +149.5 (*c* 0.50, $CHCl_3$); IR (neat) 3445, 2927, 2854, 1574, 1456, 1356, 1174, 1072, 946, 790, 627 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.13 – 1.33 (24H, m), 1.55 – 1.62 (5H, m), 1.74 – 1.78 (2H, m), 1.97 (1H, t, *J* = 11.7 Hz), 2.19 – 2.25 (2H, m), 2.89 (6H, s), 3.56 – 3.62 (1H, m), 3.90 (2H, s), 3.99 (2H, t, *J* = 6.3 Hz), 4.15 – 4.23 (1H, m), 4.28 (1H, d, *J* = 11.2 Hz), 7.20 (1H, d, *J* = 7.8 Hz), 7.52 – 7.60 (2H, m), 7.27 (2H, d, *J* = 6.3 Hz), 8.59 (1H, d, *J* = 8.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.7, 25.2, 25.4, 26.3, 28.6, 28.7, 28.9, 29.0, 29.1, 29.2 (×2), 29.7, 34.9, 38.8, 43.5, 44.0, 45.4, 61.1, 65.8, 70.9, 72.1, 74.4, 94.7, 115.5, 119.5, 123.0, 128.6, 129.8, 129.9, 130.4, 131.4 (×2), 131.5, 151.7; HRMS (ESI) m/z: $[M+Na]^+$; Calcd for $C_{34}H_{53}O_5NCl_4S$ 750.2296; Found 750.2290.

Dansyl derivative **12**

Diol **23** (5.00 mg, 6.87 μmol) was dissolved in THF (0.10 mL) and SO_3·Py complex (8.70 mg, 55.0 μmol) was added . After the mixture was stirred for 90 min, SO_3·Py complex (4.30 mg, 27.5 μmol) was added. After another 15 min, saturated $NaHCO_3$ was added and the suspension stirred for 2 h. The mixture was filtered through a silica plug, eluting with CH_2Cl_2:MeOH = 3:1. The residue was concentrated and purified by silica gel column chromatography (MeOH:CH_2Cl_2 = 5:95 then 30:70) to give dansyl derivative **12** (5.40 mg, 6.04 μmol, 88%) as a colorless oil: $[\alpha]_D^{23}$ +191.8 (*c* 0.22, MeOH); IR (neat) 3420, 2928, 2854, 1652, 1558, 1507, 1457, 1356, 1233, 1175, 1007, 792, 628 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 0.94 – 1.03 (6H, m), 1.21 – 1.40 (19H, m), 1.60 – 1.65 (2H, m), 1.73 – 1.82 (2H, m), 2.10 – 2.20 (1H, m), 2.22 – 2.27 (2H, m), 2.89 (6H, s), 3.97 (2H, t, *J* = 6.3 Hz), 4.14 – 4.23 (1H, m), 4.30 (2H, s), 4.35 – 4.42 (1H, m), 4.55 (1H, d, *J* = 10.7 Hz), 7.31 (1H, d, *J* = 7.8 Hz), 7.60 – 7.65 (2H, m), 8.21 – 8.26 (2H, m), 8.65 (1H, d, *J* = 8.8 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 25.8, 26.3, 26.4, 27.4, 29.5, 29.6, 30.0, 30.1, 30.2, 30.3, 31.7, 34.5, 35.6, 37.4, 43.5, 45.1, 45.9, 61.7, 62.1, 72.3, 75.5, 81.5, 91.3, 104.3, 116.9, 120.6, 124.3, 129.7, 131.2 (×2), 131.6, 132.6, 132.9, 153.3; HRMS (ESI) m/z: $[M–2H]^{2-}$; Calcd for $C_{34}H_{51}O_{11}NCl_4S_3$ 442.5702; Found 442.5695.

Biological Activity Test

Ten hatched brine shrimp, in ~4.95 mL of autoclaved seawater, were added to each well (12 well plate, Falcon) containing different concentrations of CSLs in 50 μL of EtOH to make a total volume of 5 mL. Then, incubated samples for 24 h (L/D 12 h:12 h, 25 ˚C). After incubation, the numbers of live and dead brine shrimp was counted.

Samples and controls (50 μL of EtOH dose wells) were tested duplicate. [42]

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number.

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Author Contribution Statement

Taiki Umezawa: Research design, structure identification and writing/reviewing/editing the draft

Takeshi Maeda: Synthesis of compounds

Takuya Akiyama: Synthesis of compounds

Nurcahyo Iman Prakoso: Synthesis of compounds

Jakia Jerin Mehjabin: Toxicity assay

Tatsufumi Okino: Toxicity assay and revision of the manuscript

Fuyuhiko Matsuda: Revision of the manuscript.

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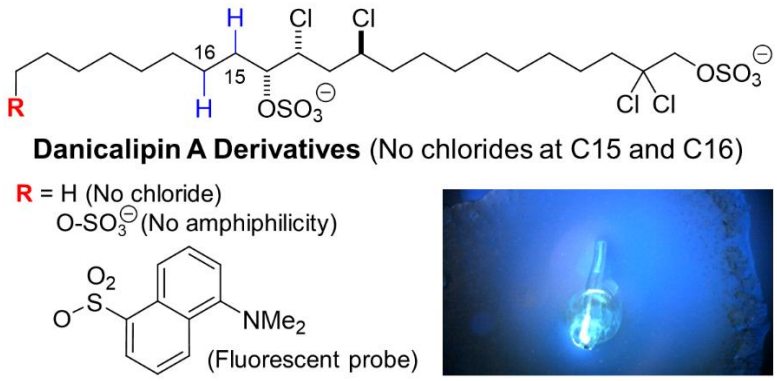

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