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Absolute Configuration of Chlorosulfolipids from the Chrysophyta *Ochromonas danica*

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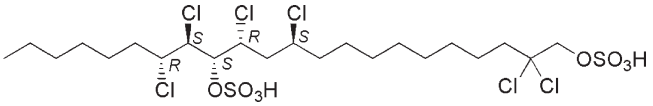

We isolated eight chlorosulfolipids (**1**−**8**) from the chrysophyta *Ochromonas danica* (IAM CS-2), including five new chlorosulfolipids (**2**−**5**, **8**). The planar structures of all the compounds were elucidated by 1D and 2D NMR and ESI-MS/MS analyses. We determined the relative configuration of seven chlorosulfolipids (**1**−**7**), including the most commonly known chlorosulfolipid, 2,2,11,13,15,16-hexachlorodocosane-1,14-disulfate (**1**), by *J*-based configuration analysis (JBCA). The absolute configuration of each compound was determined using a modified Mosher's method after chemical degradation. 2,2,11,13,15,16-Hexachloro-14-docosanol-1-sulfate (**2**) was the most toxic to brine shrimp (*Artemia salina*) larvae (LC_{50} 0.27 μg/mL). Compounds **1** and **4**−**8** were less toxic (LC_{50} 2.2−6.9 μg/mL). Compound **3** was not toxic at 30 μg/mL.

Introduction

Chlorosulfolipids (CSLs) are complex polychlorinated structures containing a sulfate group. CSLs are known to have cytotoxic activity. The chemistry of CSLs has attracted considerable attention among synthetic chemists. Recently, there has been considerable effort to synthesize CSLs.[1] The first complete synthesis of a CSL from mussels was reported in 2009.[1c] Marine chlorosulfolipid cytotoxins were first isolated from mussels a decade ago,[2] although the toxin is most likely produced by microalgae. For example, CSLs were first found in the chrysophyta *Ochromonas danica* in 1967.[3] The CSLs in *O. danica* constitute 15% of the total lipids and 3% of the dry weight of heterotrophically grown stationary phase cells. These CSLs have an interesting structure, consisting of polychlorodocosane-1,14-disulfates and polychlorotetracosane-1,15-disulfates with up to six chlorine atoms.[3d] The primary CSL produced by *O. danica* is 2,2,11,13,15,16-hexachlorodocosane-1,14-disulfate.[3d] The planar structures of these CSLs, including six minor compounds, were characterized based on infrared spectroscopy and mass spectrometry after isolation of chemically degraded mixture products.[3a] However, the lipids could not be isolated due to their high polarity and viscosity. Furthermore, the configuration of these CSLs is unknown, with the exception of simple compounds, including a nonchlorinated sulfolipid and a monochlorosulfolipid. Thus, the CSLs from *O. danica* are currently not viable targets for synthesis.

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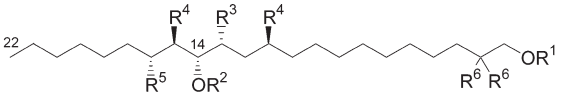
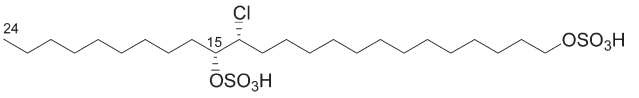
Chrysomonads in the genera *Ochromonas* and *Poterioochromonas* are known to produce chemicals that are toxic to fish[4] and invertebrates such as daphnia and rotifers.[5] These toxins also cause inhibition of bacterial growth and lysis of mammalian erythrocytes.[6] However, pure CSLs have yet to be isolated from *O. danica*. Therefore, it is unclear whether CSLs are responsible for the toxic effects observed above. Malhamensilipin A is another chlorosulfolipid, isolated from the chrysophyta *Poterioochromonas malhamensis*.[7] Malhamensilipin A inhibits the tyrosine kinase. However, its configuration is unknown.

We report the successful isolation, NMR assignment, and determination of the relative and absolute configuration of CSLs **1**−**8** from *O. danica*, in which **2**−**5** and **8** are new compounds. In addition, we tested the toxicity of these CSLs to brine shrimp (*Artemia salina*).



	R^1	R^2	R^3	R^4	R^5	R^6
1	SO_3H	SO_3H	Cl	Cl	Cl	Cl
2	SO_3H	H	Cl	Cl	Cl	Cl
3	H	H	Cl	Cl	Cl	Cl
4	SO_3H	SO_3H	Cl	Cl	Cl	H
5	SO_3H	SO_3H	Cl	Cl	H	H
6	SO_3H	SO_3H	Cl	H	H	H



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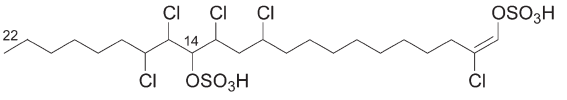

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Results and Discussion

We cultured *O. danica* (IAM CS-2) in O medium. The contents of the cells were extracted with MeOH and EtOAc. The extracts were combined and partitioned with hexane, chloroform, ethyl acetate, butanol, and H_2O. The EtOAc soluble fraction (4.0 g) was purified by bioassay-guided fractionation using brine shrimp.[8] Using a stepwise combination of silica gel and flash ODS column chromatography and RP-HPLC, we collected the following compounds: **1** (195.9 mg), **2** (9.0 mg), and **3** (2.2 mg). The BuOH soluble fraction was purified in a similar manner to yield compounds **4** (8.0 mg), **5** (5.0 mg), **6** (113.7 mg), and **7** (23.7 mg), in addition to **1** (825 mg). We also isolated compounds **8** (25.1 mg) and **1** (1 g) from the $CHCl_3$ soluble fraction.

TABLE 1. ^{13}C and ^1H NMR Data for 1

	δ_C, mult.[a]	δ_H, mult. (J in Hz)[b]
1	75.4 CH_2	4.29 s
2	91.3 C	
3	45.0 CH_2	2.24 m
4	25.7 CH_2	1.64 m
5	30.0[d] CH_2	1.34[c] m
6	30.4[e] CH_2	1.34[c] m
7	30.3[e] CH_2	1.34[c] m
8	30.0[d] CH_2	1.34[c] m
9[h]	27.4 CH_2	1.45[d] m
9[l]		1.55[e] m
10	39.8 CH_2	1.77[f] m
11	62.2 CH	4.19 m
12[h]	45.4 CH_2	2.08 ddd (1.2, 11.1, 15.6)
12[l]		2.51 ddd (1.2, 11.1, 15.6)
13	62.3 CH	4.87 br d (11.1)
14	80.8 CH	4.53 br d (10.2)
15	68.4 CH	4.44 dd (1.1, 10.2)
16	63.3 CH	4.73 br d (10.2)
17[h]	33.5 CH_2	1.70[f] m
17[l]		1.94 m
18[h]	27.6 CH_2	1.45[d] m
18[l]		1.55[e] m
19	30.0[d] CH_2	1.34[c] m
20	32.8 CH_2	1.29[f] m
21	23.6 CH_2	1.31[f] m
22	14.4 CH_3	0.89 t (6.9)

[a]MeOH-d_4, 150 MHz. [b]MeOH-d_4, 600 MHz. [c]Overlapped. [d]Interchangeable. [e]Interchangeable. [f]Overlapped. Chemical shifts were assigned by interpretation of HSQC data.

The negative ESIMS of **1** exhibited the $[M + Na - 2H]^-$ ions at m/z 727, 729, 731, 733, 735, and 737 (50:100:84:40:10:1). The molecular formula of **1** was $C_{22}H_{40}Cl_6O_8S_2$, established by negative ion HRESIMS (m/z 727.0039 $[M - Na - 2H]^-$, calcd for $C_{22}H_{38}Cl_6NaO_8S_2$, 727.0037). The mass spectrum of **1** is very similar to that of the CSL reported by Darsow et al.[9] They reported the structure of a new tetrachlorosulfolipid based on MS^n analyses of an unpurified organic extract using matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry. Although the molecular formula ($C_{20}H_{38}Cl_4O_{12}S_3$) reported in the earlier study is different, the similar spectra between these two molecules suggest that the CSL detected by Darsow et al.[9] may be the same as compound **1**. In the previous study, the authors reported different isotopic ratios for sodium and potassium adducts in the spectrum of the unpurified extract. It is difficult to compare between the studies as the compound was not isolated in the earlier study. However, our analysis of purified samples of compound **1** revealed an unambiguous isotopic mass fragment pattern consisting of six chlorine atoms (Figure S10 in Supporting Information). In addition, the NMR and MS/MS analyses support this arrangement (described below). The IR spectra at 3480 and 1250 cm^{-1} were characteristic of hydroxy and sulfate groups, respectively. The ^1H NMR (Table 1) spectrum of **1** revealed the presence of one methyl triplet (δ_H 0.89), five methines (δ_H 4.19, 4.44, 4.53, 4.73, 4.87), one methylene singlet (δ_H 4.29), and multiple methylenes (δ_H 1.29−2.51). Carbons bearing six chlorines and two sulfate esters were assigned as follows in the ^{13}C

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NMR spectrum: four chloromethines (δ_C 62.2, 62.3, 63.3, 68.4), one oxygenated methine (δ_C 80.8), one oxygenated methylene (δ_C 75.4), and a dichloro-substituted quaternary carbon (δ_C 91.3). A detailed analysis of the DQF-COSY and HMBC spectra of **1** revealed three partial structures (Figure 1) and three sp^3 methylenes. The ESI-MS/MS data confirmed the length of the methylene chains between the three parts of the molecule (Figure 2). Thus, the planar structure of **1** was 2,2,11,13,15,16-hexachlorodocosane-1,14-disulfate.

Although Elovson and Vagelos[3e] reported the same structure as **1**, they did not isolate **1** as a pure compound and did not discuss its configuration. The relative configuration of the five chiral centers was assigned using JBCA (*J*-based configuration analysis).[10] This method relies on the extensive use for ^3J(H,H) and $^{2,3}J$(C,H) coupling constants, in combination with NOE or ROE data. This technique has been widely applied to elucidating the relative configuration of various compounds featuring acyclic chains bearing substituents such as hydroxy, alkoxy, and methyl groups, halogens, and even nitrogen.[2,11] In particular, we interpreted the substitution of chlorine in **1** in the same manner as oxygen, an electron negative substituent.[2] The substitution for chlorine was proved in a synthetic study that documented the configuration of a CSL from mussels.[2c] Homonuclear coupling data were obtained from the ^{1}H NMR spectrum. The heteronuclear coupling constants were measured from the HETLOC[12] and *J*-IMPEACH-MBC[13] spectra and the gradient-selected *J*-HMBC experiment[14] (Figure 3).

At the C11−C12 axis, the *anti* configuration of H11−H12^h was inferred from the large coupling constant between H11 and H12^h (11.1 Hz), while H11−H12^l was *gauche* based on the small coupling constant for ^3J(H11/H12^l). In the HETLOC spectrum, small coupling constants for ^3J(C10/H12^h) (+3.0 Hz), ^3J(C10/H12^l) (+1.0 Hz), and ^3J(H11/C13) (+2.1 Hz) suggested *gauche* conformations for C10−H12^h, C10−H12^l, and H11−C13. A large coupling constant value for ^2J(C11/H12^h) (−7.2 Hz) was evidence for a *gauche* relationship at Cl11/H12^h. For the C12−C13 axis, we deduced *gauche* and *anti* conformations for the H12^h/H13 and H12^l/H13, respectively, based on the proton−proton coupling (H12^h/H13 1.2 Hz; H12^l/H13 11.1 Hz). Analysis of the HETLOC spectrum revealed a small coupling for ^2J(H12^h/C13) (+1.2 Hz) and a large coupling for ^2J(H12^l/C13) (−7.8 Hz). This is evidence for *anti* and *gauche* relationships at Cl13/H12^h and Cl13/H12^l, respectively. The *gauche* relationship between C11/H13 was supported by a small ^3J(C11/H13) value (+2.1 Hz). For the C13/C14 axis, the small ^3J(H13/H14) value (<2 Hz) indicated a *gauche* relationship between H13 and H14. Unfortunately, the HETLOC spectrum did not contain any peaks related to this axis. To obtain more information, we analyzed the *J*-IMPEACH-MBC spectrum. Using this method, we were able to observe the corresponding peaks, ^nJ(C,H). However, the values were below the limit of digital resolution (4 Hz). ^2J(C,H) values below 4 Hz cannot be used to assign either "small" or "medium" values in a disubstituted system.[10] Finally the gradient-selected *J*-HMBC[14] yielded small ^3J(C,H) and ^2J(C,H) values. The small value (0.4 Hz) for ^3J(C12/H14) suggested that C12/H14 was *gauche*. The small value for ^2J(C13/H14) (3.3 Hz) indicated an *anti* conformation at Cl13/H14. Furthermore, the lack of the NOESY correlation between H12 and H15 confirmed the *anti* conformation at C12/C15. At the C14−C15 bond, a large coupling constant (10.2 Hz) for ^3J(H14/H15) suggested H14/H15 is arranged in the *anti* configuration. The large values for ^2J(C14/H15) (±6.3 Hz) and ^2J(H14/C15) (±6.4 Hz) from the *J*-IMPEACH-MBC spectrum are evidence for *gauche* conformations at O14/H15 and Cl15/H14. The lack of NOESY correlation at H13/H16 revealed an *anti* relationship between C13 and C16. In addition, the values for ^3J(C13/H15) (3.2 Hz) and ^3J(H14/C16) (3.5 Hz), obtained using the gradient-selected *J*-HMBC, supported this conformation. For the C15−C16 bond, the *gauche* relationship at H15/H16 was inferred due to the small value (1.1 Hz) for ^3J(H15/H16). From the *J*-IMPEACH-MBC, ^3J(H15/C17) (±5.3 Hz) and ^2J(H15/C16) (±6.4 Hz) suggested an *anti* conformation between H15 and C17 and a *gauche* conformation between H15 and Cl16, respectively. A small value for ^2J(C15/H16) (±0.3 Hz) indicated an *anti* relationship between Cl15 and H16. Therefore, both the *J* values and the spatial data allowed the unambiguous assignment of the relative configuration of the five chiral carbons in **1**.

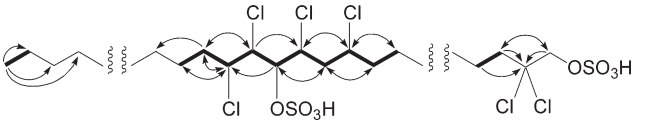

FIGURE 1. Selected DQF-COSY and HMBC correlations of **1**.

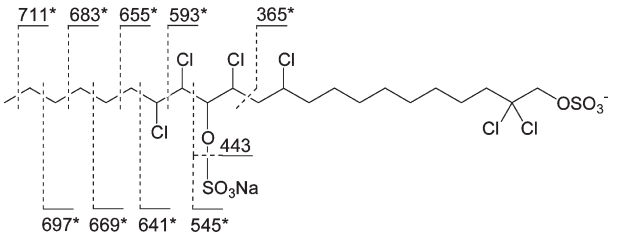

FIGURE 2. ESI-MS/MS fragment ions of **1** (*, −H).

The absolute configuration for **1** was determined using a modified Mosher's method after hydrolysis[3e] of **1** to obtain the corresponding alcohol (**1a**). Treatment of the diol with (*R*)-(−)- and (*S*)-(+)-MTPA chloride gave 1,14-(*S*)- and (*R*)-MTPA diesters (**1b** and **1c**, respectively) of **1a**. Each of the chemical shifts of ^{1}H NMR for **1b** and **1c** were assigned using ^{1}H−^{1}H COSY data. The chemical shift differences ($\Delta\delta = \delta_S - \delta_R$) are shown in Figure 4. The $\Delta\delta$ values for H10−H13 were negative. In contrast, the values for H15, H16, and H17 were positive, suggesting that C14 is arranged in the *S* configuration. Given this, the absolute configurations of **1** were assigned as 11*S*, 13*R*, 14*S*, 15*S*, and 16*R*.[1d]

Compound **2** was a colorless oil. The molecular formula of **2** was $C_{22}H_{40}Cl_6O_5S$, established by negative HRESIMS ($[M - H]^-$ *m*/*z* 625.0641, calcd for $C_{22}H_{39}Cl_6O_5S$ *m*/*z*

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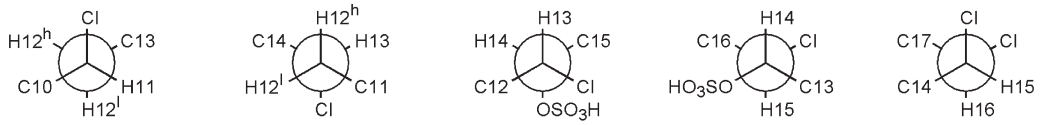

^3J(H11,H12^h) 11.1 Hz	^3J(H12^h,H13) 1.2 Hz	^3J(H13,H14) < 2 Hz	^3J(H14,H15) 10.2 Hz	^3J(H15,H16) 1.1 Hz
^3J(H11,H12^l) 1.2 Hz	^3J(H12^l,H13) 11.1 Hz	^3J(H13,C15) n. s.[b]	^3J(C13,H15) 3.2 Hz[c]	^3J(C14,H16) 0.3 Hz[c]
^3J(C10,H12^h) +3.0 Hz[a]	^3J(C11,H13) +2.1 Hz[a]	^3J(C12,H14) 0.4 Hz[c]	^3J(H14,C16) 3.5 Hz[c]	^3J(H15,C17) 5.3 Hz[d]
^3J(C10,H12^l) +1.0 Hz[a]	^2J(H12^h,C13) +1.2 Hz[a]	^2J(C13,H14) 3.3 Hz[c]	^2J(C14,H15) 6.3 Hz[d]	^2J(C15,H16) 0.3 Hz[c]
^3J(H11,C13) +2.1 Hz[a]	^2J(H12^l,C13) -7.8 Hz[a]	^2J(H13,C14) n. s.[b]	^2J(H14,C15) 6.4 Hz[d]	^2J(H15,C16) 6.4 Hz[d]
^2J(C11,H12^h) -7.2 Hz[a]				

FIGURE 3. ^3J(H,H), ^3J(C,H), and ^2J(C,H) for the C11 to C16 portion of **1**. [a]Measured by HETLOC. [b]The cross peak was not separated on the *J*-IMPEACH-MBC spectrum and was not obtained from the HETLOC spectrum. [c]Measured by the gradient-selected *J*-HMBC[14] (absolute value). [d]Measured by *J*-IMPEACH-MBC (absolute value).

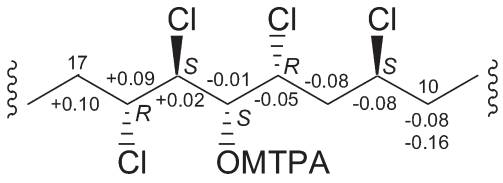

FIGURE 4. The $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained from 1,14-di-(*S*)- and (*R*)-MTPA esters (**1b** and **1c**, respectively) of **1a**.

625.0649) and the ratio of intensity of the isotope peaks (50:100:83:37:8:1). The IR spectrum indicated the presence of a hydroxy group (3451 cm^{-1}) and sulfate ester (1234 cm^{-1}). The ^{1}H NMR and ^{13}C NMR (Table 2) spectra were similar to those for **1**, with the exception of H14 and C14 (δ_H 3.76 and δ_C 75.5, respectively, versus δ_H 4.49 and δ_C 80.8 in **1**). Given this, we concluded that **2** contained a hydroxy group, substituted in place of the 14-sulfate in **1**. Analysis of the DQF-COSY and HMBC spectra of **2** revealed three partial structures: Me−$(CH_2)_2$−, −$(CH_2)_3$CHClCHClCH(OH)CHClCH$_2$CHClCH$_2$CH$_2$−, and − $(CH_2)_3CCl_2CH_2OSO_3H$ (Figure S16 in Supporting Information). The ESI-MS/MS data confirmed the length of methylene chains between the three structures (Figure 5). Thus, the planar structure of **2** was 2,2,11,13,15,16-hexachloro-14-docosanol-1-sulfate.

We used JBCA to determine the relative configuration of the five chiral centers of **2** by measuring $^3J_{H,H}$ and $^{2-3}J_{H,C}$ from the ^{1}H NMR data, the HETLOC and *J*-IMPEACH-MBC spectra, and spatial data such as the NOESY correlation. Both the *J* and NOE data for **2** and **1** were consistent for C11−C15. However, we could not determine the configuration of the C15/C16 axis because the ^2J(C15,H16) was not obtained in **2** (Figure S18 in Supporting Information). Therefore, **2** was hydrolyzed to yield its corresponding alcohol, **2a**. The ^{1}H NMR chemical shifts and coupling constants of **2a** were identical to those of **1a**, suggesting that the relative configuration of **2** is the same as **1**. The optical rotation value and sign were similar in **2a** and **1a** (**2a**: +27.9, **1a**: +34.6), further evidence that both compounds have the same absolute configuration. On the basis of our results, compound **2** was identified as (11*S*,13*R*,14*S*,15*S*,16*R*)-2,2,11,13,15,16-hexachloro-14-docosanol-1-sulfate.

Compound **3** was a colorless oil. The molecular formula of **3** was $C_{22}H_{40}Cl_6O_2$, deduced from the HRESIMS ([M − H]$^-$ *m*/*z* 545.1079, calcd for $C_{22}H_{39}Cl_6O_2$ *m*/*z* 545.1081). The IR data revealed the presence of a hydroxy group (3451 cm^{-1}). The ^{13}C NMR data (Table 2) and 2D NMR spectra, including DQF-COSY, HSQC, and HMBC, were similar to those for **2**. However, chemical shifts at C1 (**3**: δ_C 72.6, **2**: δ_C 75.4), C2 (**3**: δ_C 95.6, **2**: δ_C 91.4), and H1 (δ_H 3.84 for **3**, δ_H 4.29 for **2**) indicated that a hydroxy group was attached to C1, in place of the sulfate group. In addition, the ^{1}H NMR spectrum for **3** matched that of **1a**. The ESIMS/MS data confirmed that the planar structure of **3** was 2,2,11,13,15,16-hexachlorodocosane-1,14-diol (Figure 5). The relative configurations of the five chiral centers were not obtained completely by JBCA (Figure S27 in Supporting Information). The ^{1}H NMR chemical shifts and coupling constants of **3** were identical to those of **1a**, suggesting that the relative configuration of **3** is the same as **1**. We compared the optical rotation of **3** (+35.9) with **1a** and **2a**. Our results suggest that **3** was (11*S*,13*R*,14*S*,15*S*,16*R*)-2,2,11,13,15,16-hexachlorodocosane-1,14-diol.

Compound **4** was isolated as a colorless oil. The molecular formula of **4**, $C_{22}H_{42}Cl_4O_8S_2$, was deduced from HRESIMS ([M + Na − 2H]$^-$, *m*/*z* 659.0824, calcd for $C_{22}H_{40}Cl_4O_8S_2Na$ *m*/*z* 659.0813). The IR spectrum of **4** indicated the presence of chlorine atoms (697−588 cm^{-1}) and sulfate esters (1235 cm^{-1}).

The chemical shifts in the ^{1}H and ^{13}C NMR (Tables 3 and 4) spectra were very similar to those of **1**, except for the four contiguous methylenes assigned between CH_2-1 and CH_2-4 (δ_C 69.2, 30.2, 26.9, 30.2 for C1−C4, respectively; δ_H 3.98, 1.65, 1.40, 1.37 for H1−H4, respectively) from the HSQC and HMBC spectra. Additional 2D NMR analysis indicated the presence of three partial structures in **4** (Figure 6). The positions of the oxygen and chlorine substituents were confirmed by ESI-MS/MS measurement (Figure 5). Thus, the planar structure of **4** was 11,13,15,16-tetrachlorodocosane-1,14-disulfate.

We also determined the relative configuration of **4** by JBCA (Figure S37 in Supporting Information). On the basis of our results, both the *J* values and the spatial data allowed the assignment of the six relative configurations (detailed analysis was described in Supporting Information).

We used a modified Mosher's method to determine the absolute configuration of **4** after hydrolysis. The chemical shift differences (Figure S38 in Supporting Information) suggested that C14 was arranged in the *S* configuration. On the basis of these data, the absolute configuration of **4** was 11*S*, 13*R*, 14*S*, 15*S*, and 16*R*.

Compound **5** was a colorless oil. The molecular formula of **5** was $C_{22}H_{43}Cl_3O_8S_2$, deduced by HR-ESIMS (*m*/*z* 627.1164 [M + Na − 2H]$^-$, calcd for $C_{22}H_{41}{}^{35}Cl_2{}^{37}ClO_8S_2Na$ *m*/*z* 627.1177) and NMR spectral data (see Tables 3 and 4). Absorption at 3481 and 1233 cm^{-1} in the IR spectrum

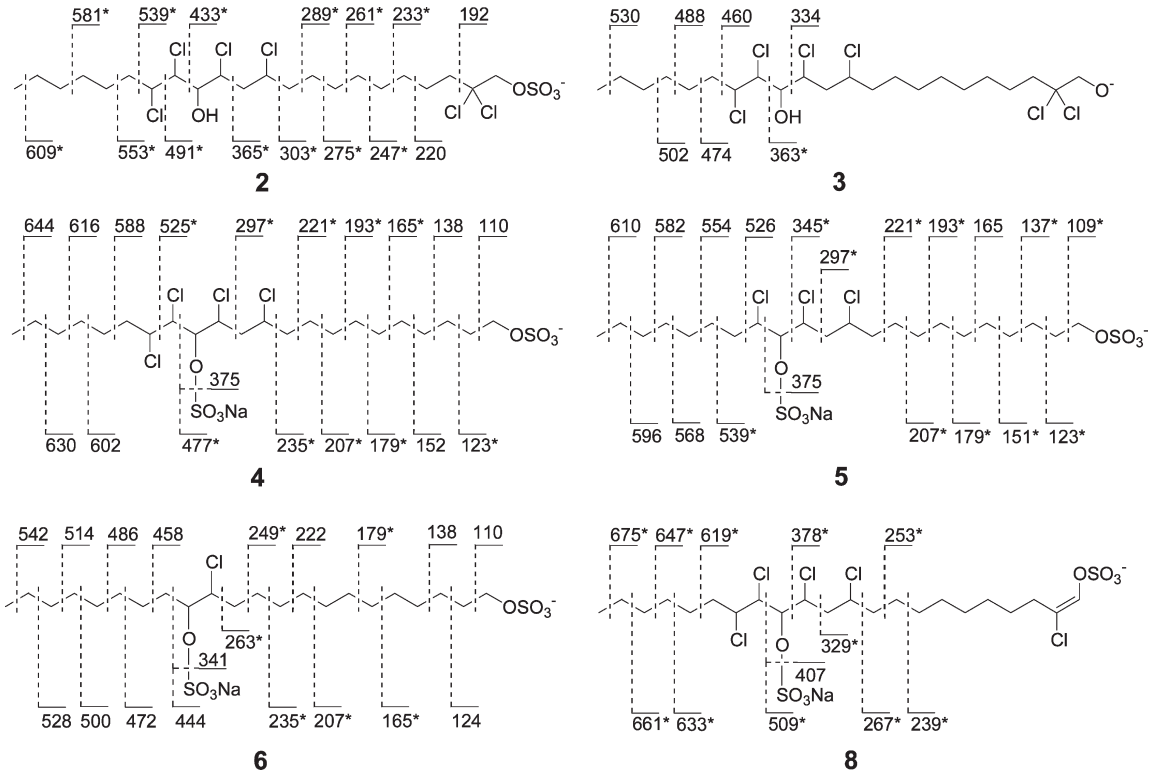

FIGURE 5. Selected ESI-MS/MS fragment ions of **2**−**6**, **8** (*, −H).

TABLE 2. ^{13}C and ^{1}H NMR Data for 2 and 3

	2				3			
	δ_C[a]	mult.	δ_H[b]	mult. (*J* in Hz)	δ_C[a]	mult.	δ_H[b]	mult. (*J* in Hz)
1	75.4	CH_2	4.29	s	72.6	CH_2	3.84	s
2	91.4	C			95.6	C		
3	45.1	CH_2	2.25	m	44.6	CH_2	2.18	m
4	25.8	CH_2	1.64	m	25.9	CH_2	1.62	m
5	30.0	CH_2	1.35	m	30.1[c]	CH_2	1.33	m
6	30.5	CH_2	1.35	m	30.4[d]	CH_2	1.33	m
7	30.3	CH_2	1.35	m	30.4[d]	CH_2	1.33	m
8	29.7	CH_2	1.35	m	30.1[c]	CH_2	1.33	m
9[h]	27.4	CH_2	1.45	m	27.4	CH_2	1.46	m
9[l]			1.56	m			1.56	m
10	39.9	CH_2	1.80	m	39.9	CH_2	1.78	m
11	62.2	CH	4.20	m	62.1	CH	4.20	m
12[h]	45.2	CH_2	1.94	ddd (2.1, 11.0, 15.1)	45.3	CH_2	1.94	ddd (2.4, 11.0, 15.0)
12[l]			2.32	ddd (2.1, 11.0, 15.1)			2.32	ddd (2.1, 11.2, 15.0)
13	63.6	CH	4.77	br d (11.0)	63.6	CH	4.77	br d (11.2)
14	75.5	CH	3.76	dd (1.2, 9.9)	75.5	CH	3.75	dd (1.3, 9.9)
15	68.5	CH	4.37	dd (2.2, 9.9)	68.5	CH	4.37	dd (2.2, 9.9)
16	63.9	CH	4.57	m	63.9	CH	4.56	m
17	32.94	CH_2	1.82	m	33.0	CH_2	1.82	m
18[h]	27.9	CH_2	1.38	m	27.9	CH_2	1.39	m
18[l]			1.63	m			1.62	m
19	29.8	CH_2	1.35	m	29.8	CH_2	1.33	m
20	32.86	CH_2	1.35	m	32.9	CH_2	1.32	m
21	23.6	CH_2	1.32	m	23.6	CH_2	1.32	m
22	14.4	CH_3	0.91	t (6.9)	14.4	CH_3	0.91	t (6.8)

[a]MeOH-d_4, 150 MHz. [b]MeOH-d_4, 600 MHz. [c]Interchangeable. [d]Interchangeable.

suggested the existence of sulfate ester functionalities. The ^{1}H and ^{13}C NMR spectra were very similar to those obtained from **4**. However, the characteristic signal for chlorine-substituted methine was not seen in the spectrum for **5**. This suggests that **5** was a deschloro derivative of **4**. The three partial structures (*n*-propyl group, trichlorinated nonyl, sulfate moiety, and propyl sulfate moiety) were deduced from the 2D NMR (HSQC, DQF-COSY, and HMBC) spectra (Figure 6). The ESI-MS/MS data confirmed the length of the methylene chains between the three structures of the molecule (Figure 5). Thus, the planar structure of **5** was 11,13,15-trichlorodocosane-1,14-disulfate.

TABLE 3. ^{1}H NMR Data for 4 and 5

	4[a]			5[a]		
	δ_H	mult.	J (Hz)	δ_H	mult.	J (Hz)
1	3.98	t	6.6	3.98	t	6.6
2	1.65	m		1.66	m	
3	1.40[c]	m		1.40[c]	m	
4	1.37[c]	m		1.32[b]	m	
5	1.31[b]	m		1.32[b]	m	
6	1.31[b]	m		1.32[b]	m	
7	1.31[b]	m		1.32[b]	m	
8	1.31[b]	m		1.32[b]	m	
9	1.46[d]	m		1.46	m	
	1.56[e]	m		1.54	m	
10	1.78	m		1.76	m	
11	4.19	m		4.20	m	
12	2.08	ddd	2.0, 11.3, 15.5	2.07	ddd	2.0, 11.2, 15.6
	2.52	ddd	2.0, 11.4, 15.5	2.53	ddd	2.0, 11.4, 15.6
13	4.87	dt	1.2, 11.4	4.93	dt	1.1, 11.4
14	4.52	dd	1.2, 10.2	4.41	dd	1.1, 9.6
15	4.45	dd	1.7, 10.2	4.12	dt	2.2, 9.6
16	4.74	dt	1.7, 10.7	1.68	m	
				2.32	m	
17	1.70	m		1.38[c]	m	
	1.94	m		1.66	m	
18	1.46[d]	m		1.32[b]	m	
	1.56[e]	m				
19	1.31[b]	m		1.32[b]	m	
20	1.30[c]	m		1.30[c]	m	
21	1.32[c]	m		1.32[b]	m	
22	0.89	t	6.9	0.90	t	7.0

[a]MeOH-d_4, 600 MHz. [b]Overlapped. [c]Overlapped. Chemical shifts were assigned by interpretation of HSQC data. [d]Interchangeable. [e]Interchangeable.

The relative configuration of **5** was determined by JBCA, as shown in Figure S48 in Supporting Information. In our results, the relative configuration of **5** was identical with **4**.

The absolute configuration of **5** was determined using a modified Mosher's method by the same procedure as **4** to obtain 1,14-(*S*)- and (*R*)-MTPA diesters (**5b** and **5c**, respectively) from the diol **5a**. The $\Delta\delta$ values ($\delta_S - \delta_R$), shown in Figure S49 in Supporting Information, indicate that the absolute configuration at C14 of **5** is *R*. Therefore, this compound was identified as (11*S*,13*R*,14*R*,15*R*)-11,13,15-trichlorodocosane-1,14-disulfate.

The molecular formula for **6** was $C_{22}H_{45}ClO_8S_2$, deduced from the ESIMS spectrum and the ratio of intensity of the isotope peaks (100:42). The ^{1}H NMR (Table 5) spectrum revealed two methine protons (δ_H 4.41 and 4.28) and a methylene proton (δ_H 3.98), as well as methylene protons (δ_H 1.93−1.29) and a methyl triplet (δ_H 0.89) in a higher field. Analyses of the ^{13}C NMR and HSQC spectra of **6** revealed the presence of two methines, 19 methylenes, and one methyl. The deshielded carbon resonance at δ_C 64.1, together with the IR absorption at 588 cm^{-1}, indicated the presence of a chlorine-substituted methine. Furthermore, IR data (ν_{max} 1228 cm^{-1}) and the deshielded methine and methylene carbon signals at δ 81.8 and δ 69.2 indicated that each carbon was connected to a sulfate ester. Analyses of the COSY and HMBC spectra allowed the assignment of the partial structures (Figure 6). The ESI-MS/MS analysis revealed that the sulfate ester connected to C14, and the chloromethine was located at C13 (Figure 5).

The relative configuration of **6** was deduced by *J* values and spatial data, indicating that the axis of C13/C14 of **6** was

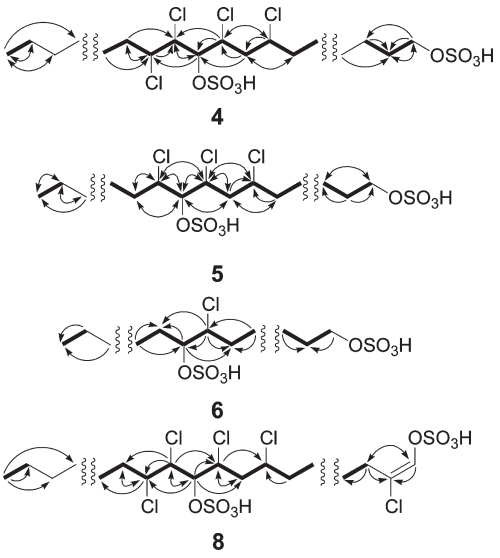

FIGURE 6. Selected DQF-COSY (bold) and HMBC (arrow curves) correlations in partial structures of **4**−**6** and **8**.

threo. Compound **6** was identical to a previously reported CSL.[3c]

We also determined the absolute configuration of **6** using a modified Mosher's method. On the basis of this analysis, we concluded that **6** was (13*R*,14*R*)-13-chlorodocosane-1,14-disulfate. The absolute configuration of **6** was also identical to the previously reported example, which was determined by comparison of optical rotation after chemical degradation to an alcohol.[3c]

Compound **7** was a colorless oil. The HRESIMS of **7** yielded a molecular formula of $C_{24}H_{49}ClO_8S_2$. The ^{1}H NMR (Table 5), ^{13}C NMR (Table 4), and IR spectra (ν_{max} 1249, 590 cm^{-1}) of **7** were similar to those of **6**. However, **7** possesses two additional sp^3 methylenes. Compound **7** was a monochlorotetracosane disulfate and an analogue of **6**. The ^{1}H−^{1}H COSY, HMBC, edited HSQC spectra, ESI-MS/MS data, JBCA, and the modified Mosher's method data all suggested that **7** was (14*R*,15*R*)-14-chlorotetracosane-1,15-disulfate.

Compound **8** was a colorless oil, isolated from the chloroform fraction. The molecular formula was $C_{22}H_{39}Cl_5O_8S_2$, based on HRESIMS ([M + Na − 2H]$^-$, *m*/*z* 691.0253, calcd for $C_{22}H_{37}Cl_5O_8S_2Na$ *m*/*z* 691.0270). The ^{1}H NMR spectrum of **8** revealed signals from one oxygenated olefinic proton (δ_H 6.72), oxygenated or chlorinated protons (δ_H 4.88, 4.75, 4.53, 4.45, 4.20), one triplet methyl proton signal (δ_H 0.90), and aliphatic methylene protons (δ_H 1.28−2.52). The ^{13}C NMR chemical shifts obtained from the edited HSQC spectrum indicated the presence of one olefinic methine (δ_C 136.2), one quaternary olefinic carbon (δ_C 124.9), one oxymethine (δ_C 80.7), four chloromethines (δ_C 68.4, 63.3, 62.4, 62.3), one methyl (δ_C 14.5), and 14 methylenes (δ_C 23.7−45.5). Analysis of the COSY and HMBC spectra allowed the assignment of the partial structures from C1−C4, C10−C18, and C19−C22 (Figure 6). The molecular formula and ESI-MS/MS data for **8** indicated that the two sulfates were connected to the oxymethine at C14 and the oxymethylene at C1 (Figure 5). The geometry of the olefin was proposed to be *E*, based on

TABLE 4. ^{13}C NMR Data for 4−8

	4[a]		**5**[a]		**6**[a]		**7**[b]		**8**[a]	
	δ_C	mult.	δ_C	mult.	δ_C	mult.	δ_C	mult.	δ_C	mult.
1	69.2	CH_2	69.2	CH_2	69.2	CH_2	69.2	CH_2	136.2	CH
2	30.2	CH_2	30.4	CH_2	30.4	CH_2	30.5	CH_2	124.9	C
3	26.9	CH_2	26.9	CH_2	26.9	CH_2	26.9	CH_2	31.9	CH_2
4	30.2	CH_2	30.0−30.7[c]	CH_2	30.4−30.8[c]	CH_2	30.3−30.8[c]	CH_2	27.7	CH_2
5	30.1−30.7[c]	CH_2	30.0−30.7[c]	CH_2	30.4−30.8[c]	CH_2	30.3−30.8[c]	CH_2	29.8−30.6[c]	CH_2
6	30.1−30.7[c]	CH_2	30.0−30.7[c]	CH_2	30.4−30.8[c]	CH_2	30.3−30.8[c]	CH_2	29.8−30.6[c]	CH_2
7	30.1−30.7[c]	CH_2	30.0−30.7[c]	CH_2	30.4−30.8[c]	CH_2	30.3−30.8[c]	CH_2	29.8−30.6[c]	CH_2
8	30.1−30.7[c]	CH_2	30.0−30.7[c]	CH_2	30.4−30.8[c]	CH_2	30.3−30.8[c]	CH_2	29.8−30.6[c]	CH_2
9	27.5	CH_2	27.5	CH_2	30.4−30.8[c]	CH_2	30.3−30.8[c]	CH_2	29.8−30.6[c]	CH_2
10	39.9	CH_2	40.0	CH_2	30.4−30.8[c]	CH_2	30.3−30.8[c]	CH_2	40.0	CH_2
11	62.3	CH	62.4	CH	26.7	CH_2	30.3−30.8[c]	CH_2	62.3	CH
12	45.5	CH_2	45.8	CH_2	30.4	CH_2	26.7	CH_2	45.5	CH_2
13	62.4	CH	62.3	CH	64.1	CH	30.5	CH_2	62.4	CH
14	80.8	CH	83.9	CH	81.8	CH	64.1	CH	80.7	CH
15	68.5	CH	63.2	CH	33.5	CH_2	81.8	CH	68.4	CH
16	63.4	CH	34.9	CH_2	28.1	CH_2	33.5	CH_2	63.3	CH
17	33.5	CH_2	27.6	CH_2	30.3	CH_2	28.1	CH_2	33.6	CH_2
18	27.6	CH_2	30.0−30.7[c]	CH_2	30.4−30.8[c]	CH_2	30.3−30.8[c]	CH_2	27.6	CH_2
19	30.1−30.7[c]	CH_2	30.0−30.7[c]	CH_2	30.4−30.8[c]	CH_2	30.3−30.8[c]	CH_2	29.8−30.6[c]	CH_2
20	32.9	CH_2	33.0	CH_2	33.0	CH_2	30.3−30.8[c]	CH_2	33.0	CH_2
21	23.6	CH_2	23.7	CH_2	23.7	CH_2	30.3−30.8[c]	CH_2	23.7	CH_2
22	14.4	CH_3	14.4	CH_3	14.4	CH_3	33.1	CH_2	14.5	CH_3
23							23.7	CH_2		
24							14.4	CH_3		

[a] MeOH-d_4, 150 MHz. [b]MeOH-d_4. 125 MHz. [c]Overlapped.

TABLE 5. ^{1}H NMR Data for 6−8

	6[a]			**7**[b]			**8**[a]		
	δ_H	mult.	*J* (Hz)	δ_H	mult.	*J* (Hz)	δ_H	mult.	*J* (Hz)
1	3.98	t	6.6	3.98	t	6.6	6.72	s	
2	1.64	m		1.65	m				
3	1.35[d]	m		1.38[e]	m		2.42	br t	13.5
4	1.29[c]	m		1.29[c]	m		1.52[d]	m	
							1.58[e]	m	
5	1.29[c]	m		1.29[c]	m		1.33[c]	m	
6	1.29[c]	m		1.29[c]	m		1.33[c]	m	
7	1.29[c]	m		1.29[c]	m		1.33[c]	m	
8	1.29[c]	m		1.29[c]	m		1.33[c]	m	
9	1.29[c]	m		1.29[c]	m		1.52[d]	m	
							1.58[e]	m	
10	1.29[c]	m		1.29[c]	m		1.78	m	
11	1.30[d]	m		1.29[c]	m		4.20	m	
	1.50[d]	m							
12	1.65[d]	m		1.51[d]	m		2.09	ddd	1.9, 11.3, 15.5
	1.81	m					2.52	ddd	1.9, 11.2, 15.5
13	4.28	dt	3.0, 10.4	1.64[d]	m		4.88	dt	1.0, 11.2
				1.81	m				
14	4.41	dt	3.0, 8.4	4.28	dt	3.2, 10.4	4.53	dd	1.0, 10.2
15	1.61[d]	m		4.41	dt	3.2, 8.1	4.45	dd	1.7, 10.2
	1.93	m							
16	1.36[d]	m		1.62[d]	m		4.75	br d	10.8
	1.57[d]	m		1.94	m				
17	1.29[c]	m		1.38[e]	m		1.71	m	
				1.58[d]	m		1.95	m	
18	1.29[c]	m		1.29[c]	m		1.46[d]	m	
							1.55[d]	m	
19	1.29[c]	m		1.29[c]	m		1.33[c]	m	
20	1.28[d]	m		1.29[c]	m		1.28	m	
21	1.31[d]	m		1.29[c]	m		1.32[d]	m	
22	0.89	t	7.0	1.29[c]	m		0.90	t	7.0
23				1.29[c]	m				
24				0.89	t	6.9			

[a]MeOH-d_4, 600 MHz. [b]MeOH-d_4, 500 MHz. [c]Overlapped. [d]Overlapped. Chemical shifts were assigned by interpretation of HSQC data. [e]Interchangeable.

the absence of NOESY correlation for H1/H3. The chemical shifts of this portion matched malhamensilipin A. The relative configuration of **8** could not be determined due to its decomposition.

We isolated eight CSLs, including five new compounds. The skeletons of these compounds were hexachlorodocosane (**1**−**3**), tetrachlorodocosane (**4**), trichlorodocosane (**5**), monochlorodocosane (**6**), monochlorotetracosane (**7**), and pentachlorodocosane (**8**). Although the three known compounds (**1**, **3**, and **6**) were not isolated in previous studies, their structures were identical to those we deduced from pure compounds using NMR and MS techniques. The absolute configurations of **1**−**7** were similar. On the basis of the isolated yield, we calculated that **1** and **6** comprised ∼91 and 5%, respectively, of the total CSLs. In previous reports,[3a,3f] the diol backbone of CSLs was biosynthesized from docosanoic acid or tetracosanoic acid. The diol was sulfated by phosphoadenosine phosphosulfate (PAPS) and chlorinated to the final CSLs.[3a] We hypothesize that the monochlorinated CSL **6** is an important precursor for CSLs. To synthesize **1**, chlorination likely starts in the middle of **4** and **5** and ends at C2. Compound **7** belongs to the C_{24}−CSL series, not the C_{22}−CSLs.

The preferred conformations for **1** were *gauche*, *anti*, and *gauche* for the electronegative substituents at C13/C14, C14/C15, and C15/C16, respectively. These conformational preferences are based on maximization of relative *gauche* orientations caused by the "*gauche* effect". This was attributed primarily to σ-hyperconjugation (in this case, σ_{C-H}−σ^*_{C-Cl}).[15] The *anti* conformation at C14/15 avoids steric hindrance.

Compound **2** was highly toxic to brine shrimp (*Artemia salina*) (LC_{50} 0.27 μg/mL), whereas compound **3** had no toxicity at 30 μg/mL. The remaining chlorosulfolipids (**1**, **4**−**8**) were somewhat toxic (LC_{50} 2.2−6.9 μg/mL, Table 6). On the basis of our results, we hypothesize that the toxic effect

(15) (a) Hoffmann, R. W. *Angew. Chem., Int. Ed.* **2000**, *39*, 2054–2070. (b) Rablen, P. R.; Hoffmann, R. W.; Hrovat, D. A.; Borden, W. T. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1719–1726.

TABLE 6. Brine Shrimp Toxic Activity of 1−8

compounds	LC_{50}, μg/mL
1	2.2
2	0.27
3	> 30
4	3.7
5	6.1
6	6.9
7	3.0
8	3.8

was caused by the surface-active potency of the CSL's amphiphilic molecular shape, not the number of chlorine atoms in the CSL.

Experimental Section

Culture, Extraction, and Isolation. The chrysophyta *O. danica* (IAM CS-2) was cultured at 25 °C for 1 week in aerated O medium (430 L) containing 0.1% glucose, 0.1% tryptone, 0.1% yeast extract, and 0.05% beef extract. The cells were collected by continuous centrifugation (12 000 rpm) and freeze-dried. The freeze-dried cells (115 g) were extracted (three times) with 1 L of MeOH and 1 L of EtOAc. The combined extracts (41 g) were evaporated in vacuo and extracted sequentially with *n*-hexane (1 L × 4), $CHCl_3$ (1 L × 3), EtOAc (1 L × 3), *n*-BuOH (1 L × 3), and H_2O (1 L).

The ethyl acetate fraction (4.0 g) was subjected to silica gel column chromatography, eluted with *n*-hexane−EtOAc and EtOAc−MeOH to yield six fractions (A_1−A_6). Fraction A_4 (LC_{50} 1−10 μg/mL) was subject to repeat silica gel column chromatography to yield three fractions. The EtOAc eluted fraction A_{42} was subjected to further silica gel chromatography to give eight fractions (A_{421}−A_{428}). Purification of A_{421} (eluted with *n*-hexane/EtOAc = 1:1) and A_{424} (eluted with EtOAc) was performed by RP-HPLC detected by ELSD, yielding compounds **2** (9.0 mg) and **3** (2.2 mg). The fraction A_{43} was fractionated by flash ODS column chromatography with MeOH−H_2O to yield three fractions. The first (25% MeOH) fraction was purified by RP-HPLC with a CH_3CN−H_2O gradient to produce compound **1** (195.9 mg).

The BuOH fraction (10.2 g) was applied to silica gel column chromatography eluted with *n*-hexane/EtOAc and EtOAc/MeOH to yield five fractions (B_1−B_5). The main fraction B_4 (EtOAc−MeOH 1:1) was subjected to silica gel column chromatography to yield three fractions. The EtOAc/MeOH (9:1) eluted fraction B_{41} was separated by ODS column chromatography with aqueous−MeOH to yield four fractions. Compound **1** (825 mg) was obtained from the MeOH/H_2O (4:1) fraction. From the MeOH/H_2O (3:2) fraction, we obtained compounds **1** (96.2 mg), **4** (8.0 mg), and **6** (47.9 mg) using RP-HPLC with CH_3CN/H_2O as the mobile phase, connected with a C_{30} column. The B_{42} fraction was further separated by ODS column chromatography to yield four fractions (B_{421}−B_{424}). Further purification of the 60% MeOH eluted fraction B_{422} by RP-HPLC (using MeCN−H_2O as the eluent) yielded compounds **5** (5.0 mg), **6** (65.8 mg), and **7** (23.7 mg).

The $CHCl_3$ fraction (17.1 g) was subjected to silica gel column chromatography, eluted with *n*-hexane−EtOAc and EtOAc−MeOH, to yield five fractions (C_1−C_5). The active fraction, C_3 (EtOAc−MeOH 9:1), was further subjected to silica gel column chromatography, eluted with *n*-hexane−EtOAc−MeOH, to give four fractions. The C_{31} fraction eluted with EtOAc was subjected to ODS column chromatography using MeOH−H_2O as the eluent to yield compounds **8** (25.1 mg) and **1** (1 g).

2,2,11,13,15,16-Hexachlorodocosane-1,14-disulfate (1): Colorless oil; $[\alpha]^{25}_D$ +12.8 (*c* 0.2, MeOH); IR (NaCl) ν_{max} 3480, 1250, 700, 665, 597, 585, 559 cm^{-1}; ^{13}C and ^1H NMR data are shown in Table 1; HRESIMS *m/z* 727.0039 $[M + Na - 2H]^-$, calcd for $C_{22}H_{38}Cl_6O_8S_2Na$ *m/z* 727.0037.

Hydrolysis of 1. Compound **1** (46.6 mg) was hydrolyzed at 100 °C for 60 min in a mixture of H_2O (250 μL), dioxane (500 μL), and 12.1 M HCl (750 μL). After cooling, the mixture was transferred to a separation funnel, diluted with EtOAc (100 mL), and washed three times with the same volume of H_2O. The organic phase was dried with Na_2SO_4 and evaporated in vacuo to give 2,2,11,13,15,16-hexachlorodocosane-1,14-diol (**1a**, 28.9 mg): colorless oil; $[\alpha]^{23}_D$ +34.6 (*c* 0.41, MeOH); ^1H NMR (MeOH-d_4, 400 MHz) δ 4.77 (br d, *J* = 10.6 Hz, H-13), 4.56 (m, H-16), 4.37 (dd, *J* = 2.1, 9.9 Hz, H-15), 4.20 (m, H-11), 3.84 (s, H_2-1), 3.75 (dd, *J* = 1.4, 9.9 Hz, H-14), 2.32 (ddd, *J* = 2.1, 10.6, 15.2 Hz, H-12^l), 2.18 (m, H-3), 1.94 (ddd, *J* = 2.0, 11.2, 15.2 Hz, H-12^h), 1.80 (m), 1.64 (m), 1.36 (m), 0.91 (t, *J* = 6.6 Hz, H-22).

Synthesis of the (*S*)- and (*R*)-MTPA Diesters of 1a. Compound **1a** (5.0 mg) was treated with (*R*)-(−)-MTPA chloride (5 μL) in anhydrous pyridine (200 μL). The mixture was allowed to react under static conditions at room temperature for 72 h. The reaction was quenched by addition of H_2O (3 mL). The mixture was diluted with H_2O up to 40 mL, and the aqueous solution was extracted with the same volume of $CHCl_3$. The organic phase was evaporated and dried using Ar gas. The residue was purified by preparative TLC (*n*-hexane/$CHCl_3$ = 6:4) to obtain the (*S*)-MTPA diester **1b** (3.4 mg). (*R*)-MTPA diester **1c** (2.4 mg) was obtained from **1a** (4.6 mg) and (*S*)-(+)-MTPA chloride (5 μL).

2,2,11,13,15,16-Hexachlorodocosane 1,14-(*S*)-MTPA Diester (1b): HRESIMS *m/z* 1001.1829 $[M + Na]^+$, calcd for $C_{42}H_{54}Cl_6F_6O_6Na$ *m/z* 1001.1848; ^1H NMR (MeOH-d_4, 400 MHz) δ 5.58 (H-14), 4.88 (H-13), 4.61 (H-15), 4.06 (H-11), 3.99 (H-16), 1.93[a] (H-10a), 1.77[a] (H-17), 1.67[a] (H-10b), [a] = chemical shifts were assigned using ^1H−^1H COSY spectral data.

2,2,11,13,15,16-Hexachlorodocosane 1,14-(*R*)-MTPA Diester (1c): HRESIMS *m/z* 1001.1834 $[M + Na]^+$, calcd for $C_{42}H_{54}Cl_6F_6O_6Na$ *m/z* 1001.1848; ^1H NMR (MeOH-d_4, 400 MHz) δ 5.59 (H-14), 4.93 (H-13), 4.59 (H-15), 4.14 (H-11), 3.90 (H-16), 2.01[a] (H-10a), 1.83[a] (H-10b), 1.67[a] (H-17), [a] = chemical shifts were assigned by interpretation of ^1H−^1H COSY data.

2,2,11,13,15,16-Hexachloro-14-docosanol-1-sulfate (2): Colorless oil; $[\alpha]^{22}_D$ +17.9 (*c* 0.2, MeOH); IR (NaCl) ν_{max} 3462, 1234, 754, 723, 707, 638 cm^{-1}; see Table 2 for ^1H and NMR ^{13}C NMR data; HRESIMS *m/z* 625.0641 $[M - H]^-$, calcd for $C_{22}H_{39}Cl_6O_5S_1$ *m/z* 625.0649.

Hydrolysis of 2. Compound **2** (2.0 mg) was hydrolyzed at 100 °C for 60 min in a mixture of 200 μL of H_2O, 400 μL of dioxane, and 300 μL of 12.1 M HCl. After cooling, the mixture was transferred to a separation funnel, diluted with EtOAc (50 mL), and washed with H_2O. The organic layer was dried over with Na_2SO_4 and evaporated in vacuo. The residue was purified by preparative TLC (*n*-hexane−EtOAc 3:1) to give 2,2,11,13,15,16-hexachlorodocosane-1,14-diol (**2a**): colorless oil; $[\alpha]^{23}_D$ +27.9 (*c* 0.075, MeOH); ^1H NMR (MeOH-d_4, 400 MHz) δ 4.77 (br d, *J* = 10.6 Hz, H-13), 4.56 (m, H-16), 4.37 (dd, *J* = 2.2, 9.9 Hz, H-15), 4.20 (m, H-11), 3.84 (s, H_2-1), 3.75 (br d, *J* = 9.9 Hz, H-14), 2.32 (m, H-12^l), 2.18 (m, H_2-3), 1.94 (m, H-12^h), 1.80 (m), 1.64 (m), 1.36 (m), 0.91 (m, H_3-22).

2,2,11,13,15,16-Hexachlorodocosane-1,14-diol (3): Colorless oil; $[\alpha]^{22}_D$ +35.9 (*c* 0.005, MeOH); IR (NaCl) ν_{max} 3451, 767, 673, 612 cm^{-1}; see Table 2 for ^1H and NMR ^{13}C NMR data; HRESIMS *m/z* 545.1079 $[M - H]^-$, calcd for $C_{22}H_{39}Cl_6O_2$ *m/z* 545.1081.

11,13,15,16-Tetrachlorodocosane-1,14-disulfate (4): Colorless oil; $[\alpha]^{22}_D$ +37.6 (*c* 0.04, MeOH); IR (NaCl) ν_{max} 3485, 1235, 697, 665, 630, 588 cm^{-1}; see Tables 3 and 4 for ^1H and ^{13}C NMR data, respectively; HRESIMS *m/z* 659.0824 $[M + Na - 2H]^-$, calcd for $C_{22}H_{40}Cl_4O_8S_2Na$ *m/z* 659.0813.

11,13,15,16-Tetrachlorodocosane 1,14-(*S*)-MTPA Diester (4b): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 5.58 (H-14), 4.61 (H-15), 4.06 (H-11), 3.99 (H-16).

11,13,15,16-Tetrachlorodocosane 1,14-(*R*)-MTPA Diester (4c): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 5.60 (H-14), 4.58 (H-15), 4.15 (H-11), 3.91 (H-16).

11,13,15-Trichlorodocosane-1,14-disulfate (5): Colorless oil; $[\alpha]^{22}_D$ +28.4 (*c* 0.025, MeOH); IR (NaCl) ν_{max} 3481, 1233, 723, 686, 630, 580 cm^{-1}; see Tables 3 and 4 for ^{1}H and ^{13}C NMR data, respectively; HRESIMS *m*/*z* 627.1164 [M + Na − 2H]$^-$, calcd for $C_{22}H_{41}{}^{35}Cl_2{}^{37}ClO_8S_2Na$ *m*/*z* 627.1177.

11,13,15-Trichlorodocosane 1,14-(*S*)-MTPA Diester (5b): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 5.42 (H-14), 4.83 (H-15), 4.30 (H-15), 4.09 (H-11).

11,13,15-Trichlorodocosane 1,14-(*R*)-MTPA Diester (5c): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 5.39 (H-14), 4.91 (H-15), 4.22 (H-11), 4.16 (H-16).

13-Chlorodocosane-1,14-disulfate (6): Colorless oil; $[\alpha]^{21}_D$ +26.1 (*c* 0.05, MeOH); IR (NaCl) ν_{max} 3470, 1228, 588 cm^{-1}; see Tables 4 and 5 for ^{13}C and ^{1}H NMR data, respectively; HRESIMS *m*/*z* 557.1962 [M+Na − 2H]$^-$, calcd for $C_{22}H_{43}ClO_8S_2Na$ *m*/*z* 557.1986.

13-Chlorodocosane 1,14-(*S*)-MTPA Diester (6b): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 5.27 (H-14), 4.08 (H-13), 1.79[a] (H-15), and 1.46[a] (H-12), *a*=chemical shifts were assigned using ^{1}H−^{1}H COSY spectral data.

13-Chlorodocosane 1,14-(*R*)-MTPA Diester (6c): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 5.24 (H-14), 4.03 (H-13), 1.72[a] (H-15), 1.64[a] (H-12), *a* = chemical shifts were assigned from ^{1}H−^{1}H COSY spectral data.

14-Chlorotetracosane-1,15-disulfate (7): Colorless oil; $[\alpha]^{21}_D$ +25.5 (*c* 0.04, MeOH); IR (NaCl) ν_{max} 3470, 1249, 590 cm^{-1}; see Tables 4 and 5 for ^{13}C and ^{1}H NMR data, respectively; HRESIMS *m*/*z* 585.2288 [M + Na − 2H]$^-$, calcd for $C_{24}H_{47}ClO_8S_2Na$ *m*/*z* 585.2299.

14-Chlorotetracosane 1,15-(*S*)-MTPA Diester (7b): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 5.24 (H-15), 4.02 (H-14), 1.79[a] (H-16), 1.51[a] (H-13), *a* = chemical shifts were assigned by ^{1}H−^{1}H COSY spectral data.

14-Chlorotetracosane 1,15-(*R*)-MTPA Diester (7c): ^{1}H NMR (MeOH-d_4, 400 MHz) δ 5.27 (H-15), 4.07 (H-14), 1.70[a] (H-16), 1.66[a] (H-13), *a* = chemical shifts were assigned by interpretation of ^{1}H−^{1}H COSY data.

2,11,13,15,16-Pentachlorodocos-1-ene-1,14-disulfate (8): Colorless oil; see Tables 4 and 5 for ^{13}C and ^{1}H NMR data, respectively; HRESIMS *m*/*z* 691.0253 [M + Na − 2H]$^-$, calcd for $C_{22}H_{37}Cl_5O_8S_2Na$ *m*/*z* 691.0270.

Brine Shrimp Toxicity Assay. We tested the toxicity of the CSLs using a modified method.[8] Ten hatched brine shrimp, in ~4.95 mL of seawater, were added to each well containing different concentrations of the compounds in 50 μL of EtOH or 50% EtOH to make a total volume of 5 mL. Samples and controls were tested in duplicate. We counted the numbers of live and dead brine shrimp after 24 h at 25 °C.

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Supporting Information Available: General methods, experimental procedures, detailed configurational analyses of **4**−**6**, characterization data for **4a**−**7a**, spectral data for **1**−**8**; HRESIMS, ^{1}H, ^{13}C, and 2D NMR spectra; figures of selected COSY and HMBC correlations for the partial structures of **2**, **3**, and **7**; figures of *J*-based configuration analysis of **2**−**7**; figures of the absolute configurations of **4**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.