Structure Revision and Absolute Configuration of Malhamensilipin A from the Freshwater Chrysophyte *Poterioochromonas malhamensis*

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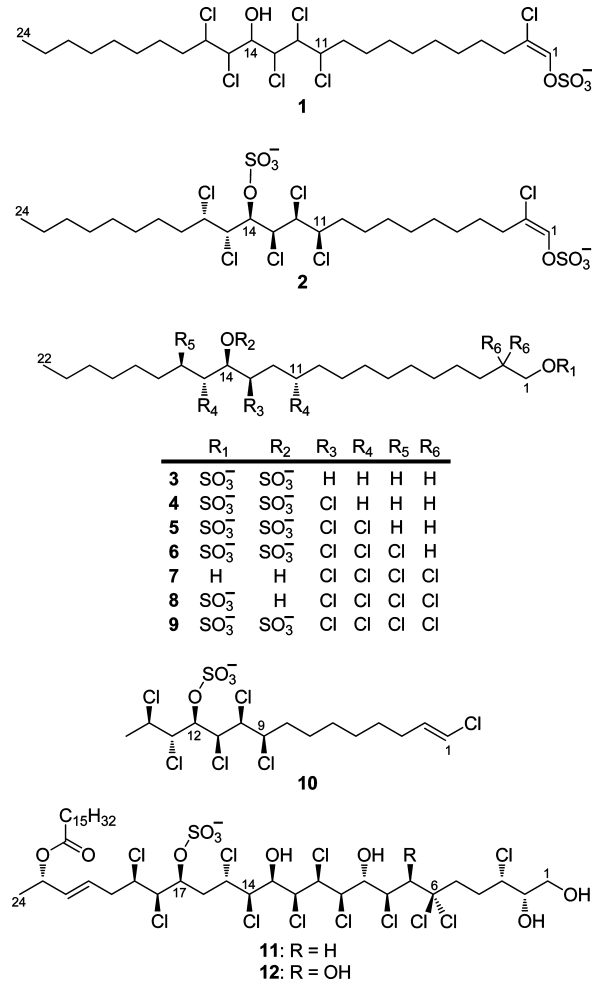
Malhamensilipin A (**2**), a bioactive chlorosulfolipid initially reported in 1994 from the freshwater alga *Poterioochromonas malhamensis*, was reinvestigated for its structural and stereochemical features. HRESIMS data revealed that **2** possesses two sulfate groups rather than the one originally reported. A combination of *J*-based configurational and Mosher's analyses led us to assign its absolute configuration as 11*R*, 12*S*, 13*S*, 14*R*, 15*S*, and 16*S*. Finally, comparison of ^{1}H and ^{13}C NMR chemical shifts with synthetic standards confirmed that malhamensilipin A (**2**) possesses a terminal double bond of *E* configuration.

In 1994 we reported the isolation and planar structure elucidation of malhamensilipin A (**1**),[1] a chlorosulfolipid metabolite from the cultured chrysophyte *Poterioochromonas malhamensis*. Compound **1** exhibited moderate protein tyrosine kinase (PTK) inhibition as well as antiviral and antimicrobial activity[1] and constituted a new member of a steadily growing family of highly chlorinated sulfolipids (Figure 1). Malhamensilipin A is closely related to the chlorosulfolipids (e.g., **3**−**9**) obtained from the freshwater alga *Ochromonas danica* over the past 40 years.[2,3] These compounds contain up to six chlorine atoms and two sulfate groups and were suspected to be structural components within cellular and flagellar membranes in *O. danica*.[4] Related natural products **10**−**12** in turn were isolated from toxic mussels and have fueled a renewed interest in this class of natural products and their possible involvement in diarrhetic shellfish poisoning.[5] These reports have stimulated efforts on the total synthesis of chlorosulfolipids and have resulted in the development of new stereoselective chlorination methodologies[6,7] as well as the total syntheses of compounds **9** and **10**.[8,9]

During our recent stereochemical studies on synthetic precursors of danicalipin A (**9**),[8] we noted that typical ^{1}H and ^{13}C chemical shift values for hydroxy-bearing methines at C-14 were δ_H 3.76−3.82 and δ_C 72.0−75.0, respectively (solvent: CD_3OD). Both of these values clearly differ from our original assignment at C-14 in malhamensilipin A (**1**, δ_H 5.02 and δ_C 77.50, CD_3OD). On the basis of these chemical shift insights and the almost ubiquitous presence of a sulfate group at equivalent positions throughout the chlorosulfolipid compound class, we predicted that malhamensilipin A actually contained a C-14 sulfate rather than a hydroxy group. To clarify this discrepancy and characterize the absolute configuration of malhamensilipin A, as well as to further explore its biological properties, we recultured the producing strain, reisolated this chlorosulfolipid, and characterized its constitutive and stereostructure as compound **2** by detailed MS and NMR analyses.

P. malhamensis was cultivated and harvested as previously reported.[1] After filtration and lyophilization, the dry cells were repetitively extracted using a mixture of CH_2Cl_2/MeOH (2:1). The crude *P. malhamensis* extract (0.4118 g) was further fractionated via normal-phase column chromatography to afford 0.1105 g (27%) of pure malhamensilipin A (**2**). HRESIMS analysis yielded an $[M + Na - H]^-$ parent ion cluster at *m/z* 753.0190 accompanied by a more intense $[M - 2H]^{2-}$ cluster at *m/z* 365.0151, both exhibiting typical isotopic patterns for a hexachlorinated compound, and in agreement with the molecular formula $C_{24}H_{41}Cl_6NaO_8S_2$. Interestingly, observation of the disulfate **2** was obtained only with freshly prepared samples of the natural product in CH_3CN, whereas MS of samples in CH_3OH afforded an unidentified hexachloro-parent ion cluster at *m/z* 649.0650, a presumed oxidation product of the originally reported monosulfated form (**1**). However, NMR



	R_1	R_2	R_3	R_4	R_5	R_6
3	SO_3^-	SO_3^-	H	H	H	H
4	SO_3^-	SO_3^-	Cl	H	H	H
5	SO_3^-	SO_3^-	Cl	Cl	H	H
6	SO_3^-	SO_3^-	Cl	Cl	Cl	H
7	H	H	Cl	Cl	Cl	Cl
8	SO_3^-	H	Cl	Cl	Cl	Cl
9	SO_3^-	SO_3^-	Cl	Cl	Cl	Cl

Figure 1. Representative chlorosulfolipids from freshwater and marine sources.

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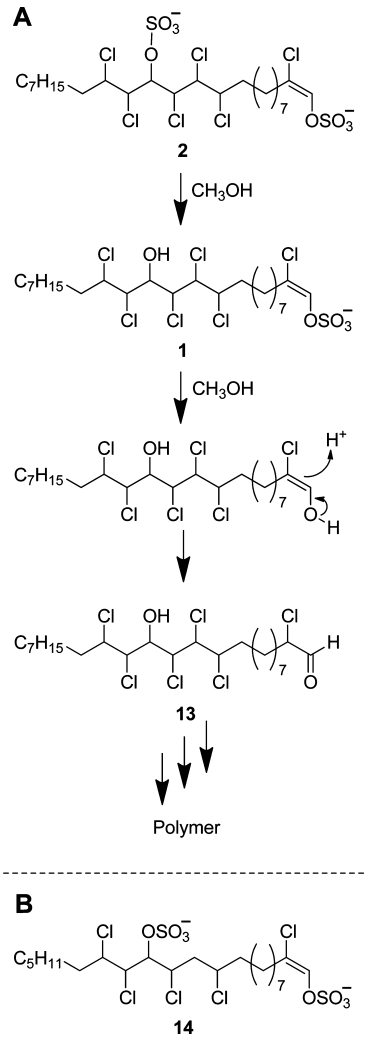

Figure 2. (A) Proposed steps in the decomposition of malhamensilipin A. (B) Structure of a recently reported and similarly unstable analogue, 2,11,13,15,16-pentachlorodocos-1-ene-1,14-disulfate (**14**).[3]

data for freshly isolated **2** in CD_3OD were in complete concordance with that initially reported for the natural product and, when acquired in DMSO-d_6, displayed proton and carbon chemical shifts supporting the presence of a sulfate group at C-14 (δ_H 4.80, δ_C 74.3, Figures S7, S8 and Table S2 in the Supporting Information). Apparently, in our previous work with malhamensilipin A, the disulfate was initially isolated and present for the NMR studies, but the labile C-14 sulfate was lost during preparation for MS analysis. However, in the current work both NMR and MS data revealed that the natural product malhamensilipin A is the 1,14-disulfate and possesses the revised structure **2**.

The lability of the C-14 sulfate in metabolite **2** was further examined through additional NMR studies. Using CD_3OD as solvent, we observed the smooth conversion of the disulfate **2** into the monosulfate species (**1**, Figure 2). HRESIMS measurements displayed in this case an $[M - H]^-$ parent ion cluster at *m/z* 651.0804, in agreement with the molecular formula $C_{24}H_{42}Cl_6O_5S$. Removal of this sulfate group substantially altered the chemical shift of H-14 (δ_H 5.02 to 4.29), causing the ^{1}H NMR spectrum of **1** (Figure S14 and Table S3, Supporting Information) to become difficult to interpret because the chloromethine protons H-11 (δ_H 4.39), H-12 (δ_H 4.42), and H-15 (δ_H 4.27), along with H-14, were almost isochronous. Compound **1** was also quite unstable, leading to the production of an insoluble film; we suspect this involves desulfation at C-1 to yield a reactive α-chloroaldehyde that might further decompose (**13**, Figure 2). Okino and co-workers recently reported the sudden decomposition of analogue **14**, presumably also through C-1 desulfation, a result that hindered their efforts to determine its relative and absolute configuration.[3]

To create a derivative of **2** with better stability as well as improved NMR characteristics, malhamensilipin A was dissolved in EtOH and treated with H_2 in the presence of 10% Pd/C, followed by hydrolysis of the resulting crude reaction product with dilute HCl.[3] These transformations afforded two derivatives, **15** and **16** (Figure 3A), in an approximate ratio of 2:1 by ^{1}H NMR analysis of the crude reaction product (Figure S19). Evidently, hydrogenation conditions favored both dechlorination at C-2 and desulfation at C-1. The more abundant derivative **15** was purified by flash chromatography and subjected to *J*-based configuration analysis (JBCA)[10] in order to determine its relative configuration (Figure 3B). The diol **16** was also used, initially in impure form and then purified subsequent to reaction, for absolute stereochemical analysis by the modified Mosher's method (Figure 3C).

Recently, we employed JBCA in the configuration analyses of danicalipin A (**9**),[8] and the applicability of this methodology to chlorinated systems such as malhamensilipin A was recently validated by combined JBCA and chemical synthesis.[7] Thus, we obtained homonuclear proton coupling constants for **15** in C_6D_6, whereas heteronuclear coupling values were measured from a combination of HETLOC and HSQMBC experiments.[12] Small homonuclear and heteronuclear *J* values were observed between methines C-11/C-12 ($^3J_{H11-H12}$ = 2.3 Hz), C-13/C-14 ($^3J_{H13-H14}$ = 1.0 Hz), and C15/C16 ($^3J_{H15-H16}$ = 1.3 Hz), a result consistent only with the *threo* rotamer **A-1**.[10] The relative configuration between C-12/C-13 ($^3J_{H12-H13}$ = 8.6 Hz) and C-14/C-15 ($^3J_{H14-H15}$ = 9.2 Hz) could not be determined using *J* values alone because these do not distinguish between the two possible rotamers (*threo* **A-3**, *erythro* **B-3** for both C-12/C-13 and C-14/C-15) when their corresponding protons possess an *anti* relationship. Fortunately, key NOE correlations were observed between H-11/H-14 and between the hydroxyl proton at C-14 and H-16 (among others), revealing a spatial proximity in agreement with only the *threo* rotamer **A-3** for C-12/C-13 and the *erythro* rotamer **B-3** for C-14/C-15, respectively. JBCA applied to the natural product malhamensilipin A (**2**) also identified two potential relative configurations at both C-12/C-13 and C-14/C-15 (Figures S6 and S13, Supporting Information), and these were the same *threo* **A-3** and *erythro* **B-3** possibilities as noted above for derivative **15**; however, not all of the NOEs described above for derivative **15** were observed in the spectra for natural product **2**.[13] Finally, modified Mosher's analysis cleanly identified that C-14 was of *R* configuration, and hence the absolute configuration of **2** was determined to be 11*R*, 12*S*, 13*S*, 14*R*, 15*S*, and 16*S*.

The final consideration in the structure of malhamensilipin A (**2**) was the geometry of the terminal double bond, originally proposed as *E* on the basis of the absence of NOE correlations between H-1 and H-3.[1] Reacquisition and analysis of NOESY and ROESY data for **2** in both CD_3OD and DMSO-d_6 failed to provide unequivocal data in support of this assignment (Tables S1 and S2, Supporting Information). Therefore, model compounds ***Z*-19** and ***E*-19** were synthesized stereoselectively as shown in Figure 4 (see Supporting Information for experimental details). Sulfation of known α,α-dichloro alcohol **17**[14] provided **18**, the substrate for a subsequent base-mediated elimination of HCl, in low but unoptimized yield. A variety of strong bases induced E2 elimination from dichloride **18**; potassium *tert*-butoxide (KO*t*-Bu) led to the predominant formation of the *Z*-isomer ***Z*-19** (ca. 5:1 ratio), whereas lithium diisopropylamide (LDA) provided exclusively the *E*-isomer (***E*-19**). The configurations of the two isomers were assigned on the basis of an observed NOE between H-1 and H-3 in ***Z*-19**, which was not detected in ***E*-19**. Comparison of ^{1}H chemical shifts of these isomeric model chlorovinyl sulfates (all in CD_3OD), which

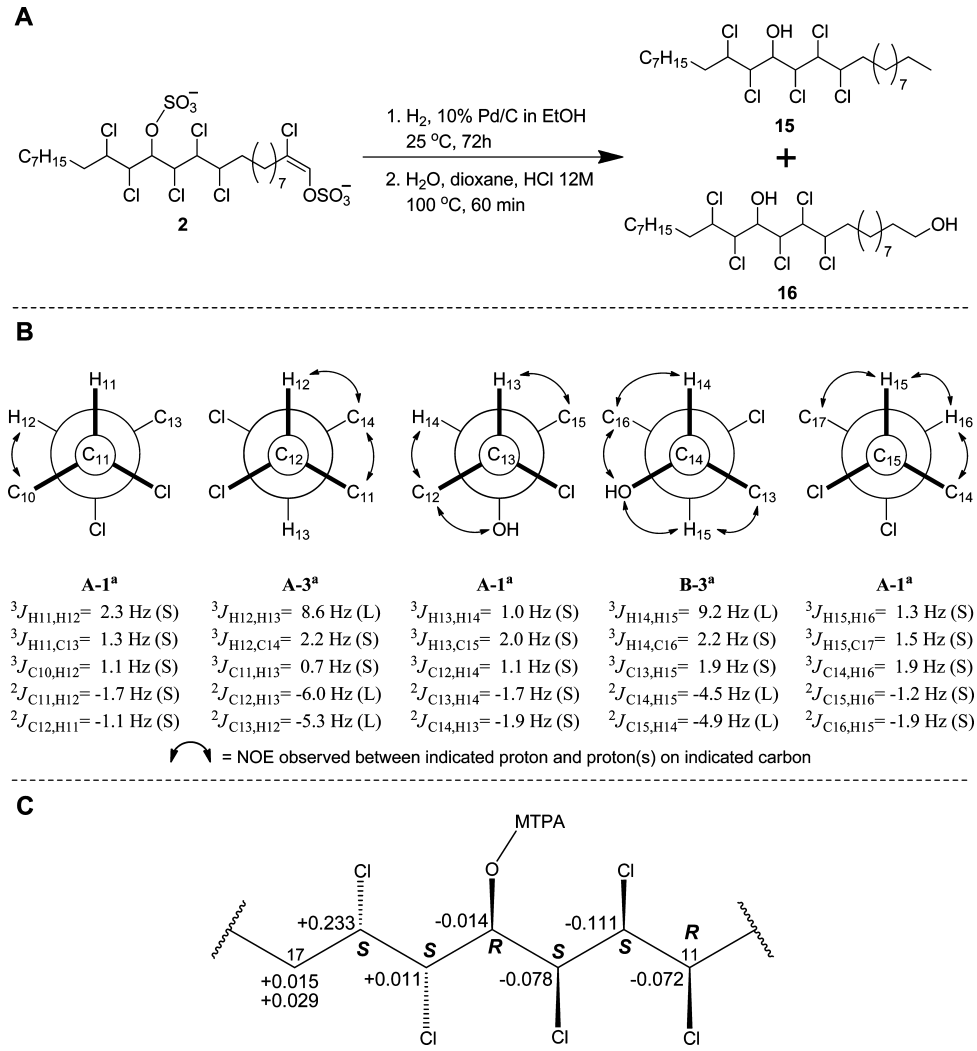

Figure 3. (A) Derivatives produced from malhamenshilipin A (**2**) for stereochemical studies. (B) *J*-Based configurational analysis (JBCA)[10] of derivative **15**. (C) $\Delta\delta^{SR}$ values (ppm) derived from the 1,14-di-(*S*)- and 1,14-di-(*R*)-MTPA esters of **16**. [a]Relative configuration and rotamer designation according to Murata and co-workers.[10]

are substantially different and therefore diagnostic, with those observed for malhamensilipin A (**2**) confirmed that the configuration of the alkene in the naturally occurring chlorosulfolipid was *E*, as originally formulated.[1]

In summary, the revised structure of malhamensilipin A (**2**) has been determined, as depicted in Figure 1, to possess two sulfate groups and an *E*-configured C-1/C-2 double bond, and by the combination of JBCA and modified Mosher's analyses, the relative and absolute configurations have been assigned. The most conserved stereocenters among the more chlorinated *Ochromonas*/*Poterioochromonas* chlorosulfolipids and mussel-derived lipid **10** are the sulfate-bearing C-14, or its positional equivalent, and the two flanking chlorine-bearing centers at C-13 and C-15. The C-12 position (or its equivalent) is variably chlorinated in these lipids, but when present, is consistent with the configuration found for compound **2**. The C-11 or equivalent position is always chlorinated, but of variable configuration; malhamensilipin A (**2**) is of opposite configuration of the majority of the *Ochromonas* compounds at this position, but has the same configuration as **10**. Finally, all other lipids in this family have a 16*R* configuration; however, malhamensilipin A (**2**) is unique in this regard, with a 16*S* chlorine-bearing center. The mechanistic biochemistry responsible for the introduction of these chlorine-bearing stereogenic centers remains a fascinating yet unknown aspect of these naturally occurring chlorosulfolipids.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Jasco P-2000 polarimeter. UV spectra were measured on a Beckman Coulter DU-800 spectrophotometer, and IR spectra were recorded on a Nicolet IR 100 FT-IR spectrophotometer. NMR spectra were collected at a ^{1}H resonance frequency of either 800 MHz (Bruker Avance 800), 600 MHz (Bruker Avance III DRX600 equipped with a 1.7 mm TCI cryoprobe), or 500 MHz (JEOL ECA500). Chemical shifts were calibrated internally to the residual signal of the solvent in which the sample was dissolved (DMSO-d_6: δ_H 2.50, δ_C 39.51; CD_3OD: δ_H 3.31; δ_C 49.5; C_6D_6: δ_H 7.16; δ_C 128.06). High-resolution mass spectra were obtained on a ThermoFinnigan MAT900XL mass spectrometer. Flash column chromatography was performed using silica gel 60 (40−63 μm, EMD). Merck aluminum-supported TLC sheets (silica gel 60 F_{254}) were used for TLC. All solvents were purchased as HPLC grade.

Culture, Extraction, and Isolation of Compounds 1 and 2. *Poterioochromonas malhamensis* (Pringsheim) Peterfi. (SAG 933-1a) was cultured and harvested as reported previously (30 L of approximately 2×10^5 cells/mL).[1] After filtration through Celite, the cells were lyophilized and the dry material extracted repeatedly with CH_2Cl_2/MeOH (2:1) to afford 0.4538 g of extract. A portion of this material (0.4118 g) was fractionated by silica gel column chromatography (12% CH_3OH in $CHCl_3$, 800 mL) until an acid-charring material was detected by TLC (33% CH_3OH in $CHCl_3$). This procedure yielded 0.1105 g (27%) of pure **2** as a colorless oil. Compound **1** was isolated from a partially decomposed sample (26 mg) of **2** by silica gel column

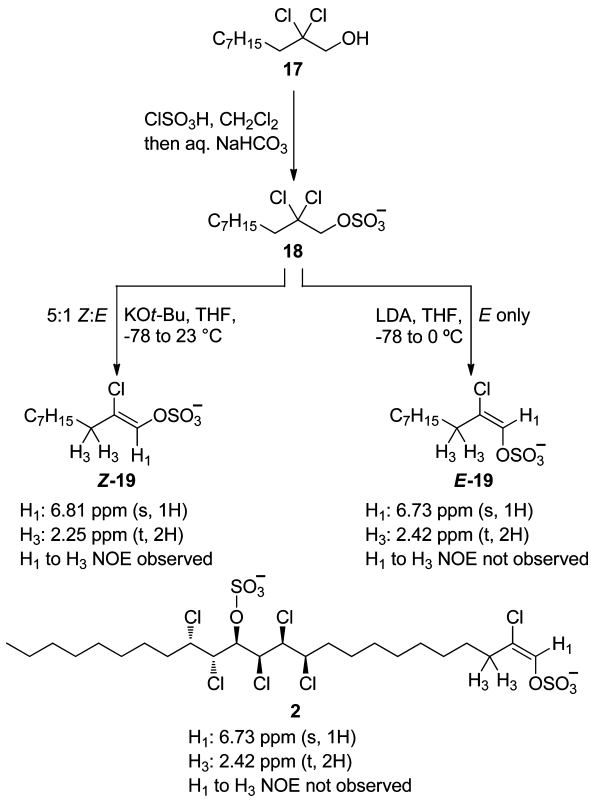

Figure 4. Synthesis of model chlorovinyl sulfates and comparison of NMR characteristics confirm that malhamensilipin A (**2**) bears an alkene of *E* geometry (data obtained in CD_3OD).

chromatography using a gradient of CH_3OH in $CHCl_3$ (5−12%). This procedure afforded pure compound **1** (3.2 mg) as a colorless oil.

Malhamensilipin A (2): specific rotation (CH_3OH), UV (CH_3OH), IR (neat), and NMR data (CD_3OD) were in accord with those previously reported;[1] ^{1}H NMR (DMSO, 600 MHz) δ 6.62 (1H, s, H-1), 5.04 (1H, ddd, J = 7.2, 3.6, 0.5 Hz, H-11), 4.80 (1H, dd, J = 9.1, 0.5 Hz, H-14), 4.76 (1H, ddd, J = 9.1, 4.8, 0.5 Hz, H-16), 4.68 (1H, dd, J = 10.0, 0.5 Hz, H-13), 4.60 (1H, dd, J = 10.0, 0.5 Hz, H-12), 4.24 (1H, dd, J = 9.6, 0.5 Hz, H-15), 2.27 (2H, t, J = 7.2 Hz, H-3), 1.83 (1H, m, H-17b), 1.82 (1H, m, H-10b), 1.81 (1H, m, H-17a), 1.74 (1H, m, H-10a), 1.48 (2H, m, H-9), 1.42 (2H, m, H-4), 1.35−1.20 (14H, m), 1.32 (2H, m, H-18), 1.25 (2H, m, H-23), 1.23 (2H, m, H-22), 0.85 (3H, t, J = 7.2 Hz); ^{13}C NMR (DMSO, 150 MHz) δ 136.8 (CH, C-1), 119.5 (CH, C-2), 74.3 (CH, C-14), 68.0 (CH, C-12), 66.8 (CH, C-13), 65.3 (CH, C-15), 62.3 (CH, C-11), 61.6 (CH, C-16), 36.8 (CH_2, C-10), 35.9 (CH_2, C-17), 31.4 (CH_2, C-22), 30.3 (CH_2, C-3), 28.94 (CH_2, C-21), 28.91 (CH_2), 28.87 (CH_2), 28.7 (CH_2), 28.6 (CH_2, C-6), 28.3 (CH_2, C-19), 28.2 (CH_2, C-5), 26.4 (CH_2, C-4), 26.2 (CH_2, C-18), 25.8 (CH_2, C-9), 22.2 (CH_2, C-23), 14.1 (CH_3, C-24); HRESIMS m/z 753.0190 (calcd for $C_{24}H_{40}Cl_6NaO_8S_2$, 753.0199).

(*E*)-2,11,12,13,15,16-Hexachloro-14-hydroxytetracos-1-en-1-yl sulfate (1): colorless oil; ^{1}H NMR (CD_3OD, 600 MHz) δ 6.75 (1H, s, H-1), 4.64 (1H, dd, J = 9.0, 1.0 Hz, H-13), 4.58 (1H, ddd, J = 7.8, 4.2, 1.0 Hz, H-16), 4.42 (1H, dd, J = 9.0, 1.8 Hz, H-12), 4.39 (1H, dd, J = 7.8, 1.8 Hz, H-11), 4.29 (1H, dd, J = 9.0, 1.0 Hz, H-14), 4.26 (1H, dd, J = 9.0, 1.0 Hz, H-15), 2.45 (2H, t, J = 7.2 Hz, H-3), 1.98 (1H, m, H-10b), 1.97 (1H, m, H-17b), 1.94 (1H, m, H-10a), 1.85 (1H, m, H-17a), 1.55 (1H, m, H-9b), 1.54 (2H, m, H-4), 1.50−1.30 (12H, m), 1.44 (1H, m, H-9a), 1.43 (2H, m, H-18), 1.35 (2H, m, H-5), 1.32 (2H, m, H-23), 1.30 (2H, m, H-22), 0.93 (3H, t, J = 7.2 Hz); ^{13}C NMR (CD_3OD, 150 MHz) δ 136.6 (CH, C-1), 125.0 (CH, C-2), 71.6 (CH, C-14), 69.7 (CH, C-12), 69.1 (CH, C-13), 66.4 (CH, C-15), 62.6 (CH, C-11), 62.4 (CH, C-16), 38.3 (CH_2, C-10), 37.5 (CH_2, C-17), 33.0 (CH_2, C-22), 31.7 (CH_2, C-3), 30.6 (CH_2), 30.5 (CH_2), 30.4 (CH_2), 30.3 (CH_2), 30.2 (CH_2), 30.1 (CH_2), 29.5 (CH_2, C-5), 27.7 (CH_2, C-18), 27.6 (CH_2, C-4), 27.4 (CH_2, C-9), 23.8 (CH_2, C-23), 14.5 (CH_3, C-24); HRESIMS m/z 651.0804 (calcd for $C_{24}H_{41}Cl_6O_5S$, 651.0800).

Derivatization of 2. A solution of malhamensilipin A (**2**) (28.5 mg, 0.038 mmol) in EtOH (5 mL) was treated with 10% Pd/C (0.5 g) and stirred under H_2 atmosphere at 25 °C for 72 h. After catalyst filtration and solvent evaporation *in vacuo*, the remaining residue was hydrolyzed at 100 °C for 60 min in a mixture of H_2O (250 μL), dioxane (500 μL), and 12 M HCl (750 μL).[3] Upon cooling to room temperature, the resulting hydrolysate was transferred into a separation funnel, diluted with EtOAc (100 mL), and washed with H_2O (3 × 100 mL). The organic layer was dried over Na_2SO_4, filtered, and then concentrated under reduced pressure to give 12.1 mg of a mixture of **15** and **16** in approximately a 2:1 ratio. A portion of this mixture (4.7 mg) was subjected to silica gel column chromatography (40% $CHCl_3$/hexanes) to afford pure compound **15** as a colorless oil (1.7 mg).

9,10,12,13,14-Pentachlorotetracosan-11-ol (15): colorless oil; ^{1}H NMR (C_6D_6, 600 MHz) δ 5.15 (1H, dd, J = 8.6, 1.1 Hz, H-13), 4.54 (1H, ddd, J = 10.4, 9.2, 1.1 Hz, H-14), 4.30 (1H, ddd, J = 9.6, 4.1, 1.3 Hz, H-16), 4.20 (1H, dd, J = 8.6, 2.1 Hz, H-12), 4.10 (1H, ddd, J = 7.9, 5.5, 2.4 Hz, H-11), 3.96 (1H, dd, J = 9.2, 1.3 Hz, H-15), 1.95 (1H, m, H-17b), 1.83 (1H, m, H-10b), 1.70 (1H, d, J = 10.6 Hz, OH), 1.68 (1H, m, H-10a), 1.47 (1H, m, H-17a), 1.46 (1H, m, H-18b), 1.33 (2H, m), 1.31−1.08 (16H, m), 1.30 (1H, m), 1.29 (2H, m), 1.251 (1H, m), 1.249 (1H, m), 1.23 (1H, m, H-9b), 1.22 (1H, m, H-18a), 1.21 (1H, m), 1.05 (1H, m, H-9a), 0.94 (3H, t, J = 6.8 Hz), 0.93 (3H, t, J = 7.2 Hz); ^{13}C NMR (C_6D_6, 150 MHz) δ 72.0 (CH, C-14), 68.14 (CH, C-13), 68.06 (CH, C-12), 65.1 (CH, C-15), 61.7 (CH, C-16), 61.6 (CH, C-11), 36.9 (CH_2, C-10), 36.5 (CH_2, C-17), 32.3 (CH_2), 32.2 (CH_2), 30.0 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.74 (CH_2), 29.71 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 27.0 (CH_2, C-18), 26.5 (CH_2, C-9), 23.11 (CH_2), 23.07 (CH_2), 14.37 (CH_3), 14.35 (CH_3); HRESIMS m/z 637.1760 (calcd for $C_{24}H_{44}Cl_5O\cdot CF_3COOH$, 637.1758).

(*S*)- and (*R*)-MTPA Diesters of 16. (*R*)-(−)-MTPA chloride (20 μL, 0.11 mmol) and DMAP (0.1 mg, 0.8 μmol) were added to a mixture of **15/16** (3.9 mg) in pyridine (200 μL) and stirred at 25 °C for 72 h, at which time the reaction was concentrated to dryness under reduced pressure. Silica gel column chromatography (40% $CHCl_3$/hexanes) yielded the (*S*)-MTPA diester of **16** as a colorless oil (1.3 mg): ^{1}H NMR (600 MHz, CD_3OD) δ 7.59 (2H, m), 7.50 (2H, m), 7.53 (3H, m), 7.43 (3H, m), 5.63 (1H, d, J = 9.6 Hz), 4.94 (1H, d, J = 9.9 Hz), 4.68 (1H, ddd, J = 7.9, 4.9, 1.9 Hz), 4.54 (1H, dd, J = 9.8, 1.7 Hz), 4.34 (1H, m), 4.29 (1H, m), 3.97 (1H, dd, J = 9.2, 1.9 Hz), 3.77 (1H, ddd, J = 9.2, 4.9, 1.6 Hz), 3.53 (3H, d, J = 1.2 Hz), 3.45 (3H, d, J = 0.4 Hz), 1.96 (1H, m), 1.85 (1H, m), 1.81 (1H, m), 1.78 (1H, m), 1.68 (2H, m), 1.46 (1H, m), 1.36 (1H, m), 1.35−1.25 (14H, m), 1.33 (2H, m), 1.299 (2H, m), 1.298 (2H, m), 1.292 (1H, m), 1.291 (2H, m), 1.25 (1H, m), 0.91 (3H, t, J = 7.2 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 167.9 (C), 166.9 (C), 133.8 (C), 133.4 (C), 131.7 (CH), 130.8 (CH), 130.3 (2CH), 129.5 (2CH), 129.03 (CH), 129.02 (CH), 128.52 (CH), 128.51 (CH), 125.9 (CF_3, d, J = 14.7 Hz), 123.7 (CF_3, d, J = 15.7 Hz), 86.4 (C), 86.2 (C), 75.2 (CH), 67.7 (CH), 67.6 (CH_2), 65.2 (CH), 64.1 (CH), 62.2 (CH), 61.5 (CH), 56.0 ($2CH_3$), 38.0 (CH_2), 37.0 (CH_2), 33.0 (CH_2), 30.477 (CH_2), 30.470 (CH_2), 30.4 (CH_2), 30.3 ($2CH_2$), 30.1 (CH_2), 29.9 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 27.4 (CH_2), 27.1 (CH_2), 26.9 (CH_2), 23.8 (CH_2), 14.5 (CH_3); HRESIMS m/z $[M + H]^+$ 973.2731 (calcd for $C_{44}H_{60}Cl_5F_6O_6$, 973.2731). Using the same procedure with (*S*)-(+)-MTPA chloride and a mixture of **15** and **16** (4.7 mg), the (*R*)-MTPA diester of **16** was prepared and isolated (silica gel column chromatography, 40% $CHCl_3$/hexanes) as a colorless oil (0.4 mg): ^{1}H NMR (600 MHz, CD_3OD) δ 7.66 (2H, m), 7.50 (2H, m), 7.49 (3H, m), 7.43 (3H, m), 5.65 (1H, d, J = 9.6 Hz), 5.02 (1H, d, J = 9.6 Hz), 4.76 (1H, ddd, J = 7.8, 5.4, 1.8 Hz), 4.53 (1H, dd, J = 9.6, 1.2 Hz), 4.35 (1H, m), 4.29 (1H, m), 4.08 (1H, dd, J = 9.6, 1.8 Hz), 3.70 (3H, s), 3.54 (1H, ddd, J = 12.0, 7.2, 1.7 Hz), 3.53 (3H, s), 1.95 (1H, m), 1.823 (1H, m), 1.821 (1H, m), 1.76 (1H, m), 1.683 (2H, m), 1.680 (2H, m), 1.40−1.22 (16H, m), 1.36 (1H, m), 1.34 (1H, m), 1.32 (2H, m), 1.31 (2H, m), 1.29 (1H, m), 1.28 (1H, m), 0.92 (3H, t, J = 7.2 Hz); ^{13}C NMR (150 MHz, CD_3OD) δ 167.6 (C), 166.9 (C), 133.8 (C), 132.5 (C), 131.6 (CH), 130.8 (CH), 130.0 (2CH), 129.5 (2CH), 128.5 (2CH), 128.3 (2CH), 124.2 (CF_3), 123.5 (CF_3), 86.4 (C), 86.3 (C), 75.2 (CH), 67.6 (CH), 67.5 (CH_2), 65.3 (CH), 64.1 (CH), 62.0 (CH), 61.6 (CH), 57.3 ($2CH_3$), 37.9 (CH_2), 37.0 (CH_2), 33.0 (CH_2), 30.5 (CH_2), 30.4 (CH_2), 30.32 ($2CH_2$), 30.28 (CH_2), 30.1 (CH_2), 29.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 27.4 (CH_2), 26.94 (CH_2), 26.92 (CH_2), 23.8 (CH_2), 14.5 (CH_3); HRESIMS m/z $[M + Na]^+$ 995.2573 (calcd for $C_{44}H_{59}Cl_5F_6NaO_6$, 995.2551).

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra in CD_3OD, DMSO-d_6, or C_6D_6 for malhamensilipin A (**2**), derivatives **1** and **15**, and 1,14-di-MPTA esters of **16**, including *J*-based configuration analyses for **2** and **15**. ^{1}H NMR, ^{13}C NMR, and NOE spectra for ***Z*-19** and ***E*-19**, as well as their preparation, are also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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