Published in final edited form as:

J Nat Prod. 2010 February 26; 73(2): 279–283. doi:10.1021/np900672h.

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

Alban R. Pereira[†], Tara Byrum[†], Grant M. Shibuya[‡], Christopher D. Vanderwal[‡], and William H. Gerwick^{†,*}

[†]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, and Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093

[‡]Department of Chemistry, University of California, Irvine, California 92697

Abstract

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 ¹H and ¹³C 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 into 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 ¹H and ¹³C 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: CD₃OD). 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, CD₃OD). Based on these chemical shift insights, and the almost ubiquitous presence of a sulfate

*To whom correspondence should be addressed. Tel: (858)-534-0578. Fax: (858)-534-0529. wgerwick@ucsd.edu.

Supporting Information Available. ¹H NMR, ¹³C NMR, and 2D NMR spectra in CD₃OD, DMSO-*d*₆ or C₆D₆ for malhamensilipin A (**2**), derivatives **1**, **15**, and 1,14-di-MPTA esters of **16**, including *J*-based configuration analyses for **2** and **15**. ¹H NMR, ¹³C 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>.

group at equivalent positions throughout the chlorosulfolipid compound class, we predicted that malhamensilipin A actually contained a C-14 sulfate rather than 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, re-isolated 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 CH₂Cl₂/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-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 C₂₄H₄₁Cl₆NaO₈S₂. Interestingly, observation of the disulfate **2** was only obtained with freshly prepared samples of the natural product in CH₃CN, whereas MS of samples in CH₃OH 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 data for freshly isolated **2** in CD₃OD 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 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 CD₃OD 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 C₂₄H₄₂Cl₆O₅S. Removal of this sulfate group substantially altered the chemical shift of H-14 (δ_H 5.02 to 4.29) causing the ¹H 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 coworkers recently reported the sudden decomposition of analogue **14**, presumably also through C-1 desulfation, a result which 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 H₂ in the presence of 10% Pd/C, followed by hydrolysis of the resulting crude reaction product with diluted HCl.³ These transformations afforded two derivatives, **15** and **16** (Figure 3A), in an approximated ratio 2:1 by ¹H NMR analysis of the crude reaction product (Figure S19). Evidently, hydrogenation conditions favored both dechlorination at C-2 as well as desulfation at C-1. The more abundant derivative **15** was purified by flash chromatography and subjected to *J*-based configuration analysis (JBICA)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 like malhamensilipin A was recently validated by combined JBCA and chemical synthesis.⁷ Thus, we obtained homonuclear proton coupling constants for **15** in C₆D₆, 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 (³*J*_{H11–H12} = 2.3 Hz), C-13/C-14 (³*J*_{H13–H14} = 1.0 Hz), and C15/C16 (³*J*_{H15–H16} = 1.3 Hz); a result consistent only with the *threo* rotamer **A-1**.¹⁰ The relative configuration between C-12/C-13 (³*J*_{H12–H13} = 8.6 Hz) and C-14/C-15 (³*J*_{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* based on the absence of NOE correlations between H-1 and H-3.¹ Reacquisition and analysis of NOESY and ROESY data for **2** in both CD₃OD 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-dichloroalcohol **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 diisopropyl amide (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 ¹H chemical shifts of these isomeric model chlorovinyl sulfates (all in CD₃OD), which 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 *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 to 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 either at a ^1H resonance frequency of 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 μ , EMD). Merck aluminum-supported TLC sheets (silica gel 60 F₂₅₄) 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 approx. 2×10^5 cells/mL).¹ After filtration through Celite, the cells were lyophilized and the dry material extracted repeatedly with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (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 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 that previously reported;¹ ^1H 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 $\text{C}_{24}\text{H}_{40}\text{Cl}_6\text{NaO}_8\text{S}_2$, 753.0199).

(E)-2,11,12,13,15,16-hexachloro-14-hydroxytetracos-1-en-1-yl sulfate (1)—colorless oil; ^1H 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 (CH₂, C-10), 37.5 (CH₂, C-17), 33.0 (CH₂, C-22), 31.7 (CH₂, C-3), 30.6 (CH₂), 30.5 (CH₂), 30.4 (CH₂), 30.3 (CH₂), 30.2 (CH₂), 30.1 (CH₂), 29.5 (CH₂, C-5), 27.7 (CH₂, C-18), 27.6 (CH₂, C-4), 27.4 (CH₂, C-9), 23.8 (CH₂, C-23), 14.5 (CH₃, C-24); HRESIMS m/z 651.0804 (calcd for C₂₄H₄₁Cl₆O₅S, 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 H₂ 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 H₂O (250 µL), dioxane (500 µL) and 12 M HCl (750 µL).³ Upon cooling to room temperature, the resulting hydrolyzate was transferred into a separation funnel, diluted with EtOAc (100 mL) and washed with H₂O (3 × 100 mL). The organic layer was dried over Na₂SO₄, 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% CHCl₃/hexanes) to afford pure compound **15** as a colorless oil (1.7 mg).

9,10,12,13,14-Pentachlorotetracosan-11-ol (15)—colorless oil; ¹H NMR (C₆D₆, 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); ¹³C NMR (C₆D₆, 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 (CH₂, C-10), 36.5 (CH₂, C-17), 32.3 (CH₂), 32.2 (CH₂), 30.0 (CH₂), 29.9 (CH₂), 29.8 (CH₂), 29.74 (CH₂), 29.71 (CH₂), 29.6 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 27.0 (CH₂, C-18), 26.5 (CH₂, C-9), 23.11 (CH₂), 23.07 (CH₂), 14.37 (CH₃), 14.35 (CH₃); HRESIMS m/z 637.1760 (calcd for C₂₄H₄₄Cl₅O.CF₃COOH, 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% CHCl₃/hexanes) yielded the (S)-MTPA diester of **16** as a colorless oil (1.3 mg): ¹H NMR (600 MHz, CD₃OD) 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); ¹³C NMR (150 MHz, CD₃OD) 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 (CF₃, d, J = 14.7 Hz), 123.7 (CF₃, d, J = 15.7 Hz), 86.4 (C), 86.2 (C), 75.2 (CH), 67.7 (CH), 67.6 (CH₂), 65.2 (CH), 64.1 (CH), 62.2 (CH), 61.5 (CH), 56.0 (2CH₃), 38.0 (CH₂), 37.0 (CH₂), 33.0 (CH₂), 30.477 (CH₂), 30.470 (CH₂), 30.4 (CH₂), 30.3 (2CH₂), 30.1 (CH₂), 29.9 (CH₂), 29.8 (CH₂), 29.5 (CH₂), 27.4 (CH₂), 27.1 (CH₂), 26.9 (CH₂), 23.8 (CH₂), 14.5 (CH₃); HRESIMS m/z [M+H]⁺ 973.2731 (calcd for C₄₄H₆₀Cl₅F₆O₆, 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% CHCl₃/hexanes) as a colorless oil (0.4 mg): ¹H NMR (600 MHz, CD₃OD) 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); ¹³C NMR (150 MHz, CD₃OD) 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 (CF₃), 123.5 (CF₃), 86.4 (C), 86.3 (C), 75.2 (CH), 67.6 (CH), 67.5 (CH₂), 65.3 (CH), 64.1 (CH), 62.0 (CH), 61.6 (CH), 57.3 (2CH₃), 37.9 (CH₂), 37.0 (CH₂), 33.0 (CH₂), 30.5 (CH₂), 30.4 (CH₂), 30.32 (2CH₂), 30.28 (CH₂), 30.1 (CH₂), 29.9 (CH₂), 29.7 (CH₂), 29.5 (CH₂), 27.4 (CH₂), 26.94 (CH₂), 26.92 (CH₂), 23.8 (CH₂), 14.5 (CH₃); HRESIMS *m/z* [*M* + Na]⁺ 995.2573 (calcd for C₄₄H₅₉Cl₅F₆NaO₆, 995.2551).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge support from NIH CA52955 and NS053398. We thank A. Jansma (UCSD) for assistance with NMR techniques and Y. Su (UCSD) for MS data acquisition. We thank the UC Irvine School of Physical Sciences and Amgen (Young Investigator Award to C.D.V.) for support of the work done at UC Irvine. G.M.S. was the recipient of a UC Irvine GAANN fellowship.

References and Notes

1. Chen JL, Proteau PJ, Roberts MA, Gerwick WH. *J. Nat. Prod* 1994;57:524–527. [PubMed: 8021653]
2. (a) Elovson J, Vagelos PR. *Proc. Natl. Acad. Sci. U.S.A* 1969;62:957–963. [PubMed: 5257016] (b) Haines TH, Pousada M, Stern B, Mayers GL. *Biochem. J* 1969;113:565–566. [PubMed: 5812095] (c) Elovson J, Vagelos PR. *Biochemistry* 1970;9:3110–3126. [PubMed: 4321366]
3. Kawahara T, Kumaki Y, Kamada T, Ishii T, Okino T. *J. Org. Chem* 2009;74:6016–6025.
4. Chen LL, Pousada M, Haines TH. *J. Biol. Chem* 1976;251:1835–1842. [PubMed: 943398]
5. (a) Ciminiello P, Fattorusso E, Forino M, Magno S, Di Rosa M, Ianaro A, Poletti R. *J. Org. Chem* 2001;66:578–582. [PubMed: 11429832] (b) Ciminiello P, Dell'Aversano C, Fattorusso E, Forino M, Di Rosa M, Ianaro A, Poletti R. *J. Am. Chem. Soc* 2002;124:13114–13120. [PubMed: 12405839] (c) Ciminiello P, Dell'Aversano C, Fattorusso E, Forino M, Magno S. *Pure Appl. Chem* 2003;75:325–336. (d) Ciminiello P, Dell'Aversano C, Fattorusso E, Forino M, Magno S, Di Meglio P, Ianaro A, Poletti R. *Tetrahedron* 2004;60:7093–7098. (e) Ciminiello P, Fattorusso E. *Eur. J. Org. Chem* 2004:2533–2551.
6. (a) Shibuya GM, Kanady JS, Vanderwal CD. *J. Am. Chem. Soc* 2008;130:12514–12518. [PubMed: 18717563] (b) Kanady JS, Nguyen JD, Ziller JW, Vanderwal CD. *J. Org. Chem* 2009;74:2175–2178. (c) Yoshimitsu T, Fukumoto N, Tanaka T. *J. Org. Chem* 2009;74:696–702. [PubMed: 19053592]
7. Nilewski C, Geisser RW, Ebert MO, Carreira EM. *J. Am. Chem. Soc* 2009;131 web release: Oct 9, 2009; doi:10.1021/ja906461h.
8. Bedke DK, Shibuya GM, Pereira A, Gerwick WH, Haines TH, Vanderwal CD. *J. Am. Chem. Soc* 2009;131:7570–7572. [PubMed: 19445461]
9. Nilewski C, Geisser RW, Carreira EM. *Nature* 2009;457:573–576. [PubMed: 19177127]
10. Matsumori N, Kaneno D, Murata M, Nakamura H, Tachibana K. *J. Org. Chem* 1999;64:866–876. [PubMed: 11674159]
11. Pereira A, Cao Z, Murray TF, Gerwick WH. *Chem. Biol* 2009;19:893–906. [PubMed: 19716479]

12. (a) Uhrin D, Batta G, Hruby VJ, Barlow PN, Kover KE. J. Magn. Reson 1998;130:155–161. [PubMed: 9515088] (b) Williamson RT, Marquez BL, Gerwick WH, Kover KE. Magn. Reson. Chem 2000;38:265–273.
13. Unlike malhamensilipin A (**2**), the presence of a hydroxy proton at δ_{H} 1.70 in derivative **15**, allowed detection of strong NOE correlations with H-12 (δ_{H} 4.20), H-15 (δ_{H} 3.96) and H-16 (δ_{H} 4.30), thus permitting an unambiguous assignment of the *erythro* rotamer **B-3** between the C-14/C-15 stereocenters.
14. This compound is known: Salgado A, Huybrechts T, De Buyck L, Czombos J, Tkachev A, De Kimpe N. Synth. Commun 1999;29:57–63.

Figure 1.
Representative chlorosulfolipids from freshwater and marine sources.

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

Figure 3.

(A) Derivatives produced from malhamenshilipin A (**2**) for stereochemical studies. (B) *J*-based configurational analysis (JBCA)¹⁰ of derivative **15**. (C) $\Delta\delta^{\text{SR}}$ values (ppm) derived from the 1,14-di-(*R*)-MTPA esters of **16**. ^aRelative configuration and rotamer designation according to Murata and coworkers.¹⁰

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 CD₃OD).