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Relative Stereochemistry Determination and Synthesis of the Major Chlorosulfolipid from *Ochromonas danica*

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In 1969, when few polychlorinated natural products were known, the groups of Haines and Vagelos independently described a series of unusual chlorinated lipids isolated from the freshwater alga *Ochromonas danica* (**1**−**4**, Figure 1).[1] These lipids contain up to

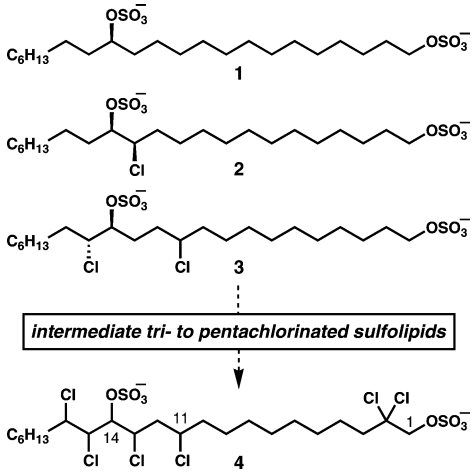

Figure 1. Docosane disulfate lipids of *Ochromonas danica*.

six chlorine atoms along carbon chains that also bear two sulfates. The gross structures of these lipids were elucidated by mass spectral analysis and degradation studies,[1a−c] and the most heavily chlorinated member was assigned the planar structure **4**. The apparent localization of these lipids in the cellular and flagellar membranes, their presence in substantial quantities (91 mol % of the polar lipids in the flagellar membrane[2]), and the lack of significant quantities of phospholipids led to speculation that these unusual compounds might play a structural role within the membranes. The presence of hydrophilic sulfate groups at both the terminus and partway down the chain makes a typical membrane bilayer morphology difficult to reconcile with the structure of these chlorosulfolipids.[1e] The extent of chlorination of the *O. danica* sulfolipids, which bear anywhere from zero to six chlorine atoms, appears to be influenced by the concentration of chloride ion in the environment.[1a] Chlorination likely occurs one halogen at a time at unactivated carbons, possibly via a radical mechanism,[1d] and the lipids are apparently formed as single stereoisomers. The relatively widespread occurrence of chlorosulfolipids in lesser quantities in other freshwater algae was proven by Mercer and Davies.[3] After further studies by the groups of Haines, Elovson, and Mercer throughout the 1970s, work on these compounds largely subsided.[4]

More recently, Slate, Gerwick, and co-workers reported the isolation and gross structural elucidation of malhamensilipin A (**5**, Figure 2), a protein tyrosine kinase inhibitor with antiviral and

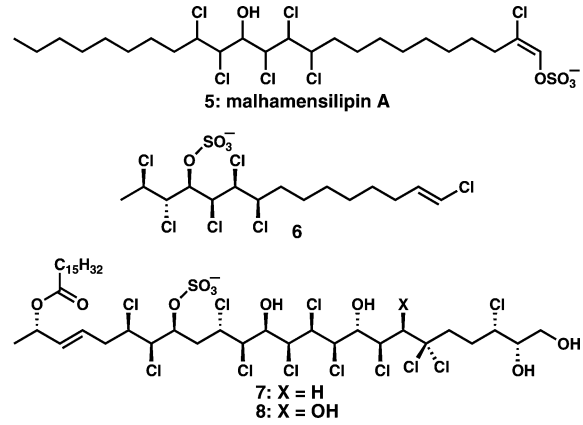

Figure 2. Other chlorosulfolipids isolated from algae and toxic mussels.

antimicrobial activity, from *Poterioochromonas malhamensis*.[5] The structure of **5** suggests that it might share a biogenesis with the lipids of *O. danica*, which is further supported by the close relationship of these two algae species. Finally, since 2001, the group of Ciminiello and Fattorusso has described the isolation and structural elucidation of **6**−**8** from toxic Adriatic mussels; these lipids appear to be a causative agent of Diarrhetic Shellfish Poisoning.[6] As a class, the chlorosulfolipids were largely ignored by synthetic chemists until recently. We reported our initial study of the syntheses of polychlorinated motifs relevant to these targets in 2008,[7] followed shortly thereafter by the elegant epoxide chlorination work of the Tanaka group.[8] Shortly before submission of this manuscript, Carreira and co-workers described their synthesis of racemic lipid toxin **6**, which constitutes the first synthesis of any member of this class and provides insight into the potential for unusual reactivity in polychlorinated alkanes.[9]

The close structural similarities of **4**−**6** and their collective differences from other known natural products suggested the possibility of stereochemical conservation among these lipids. The elucidation of the relative stereochemistry of the (sulfated) chlorohydrin motifs in **2** and **3** by Haines[1b] further suggested stereochemical homogeneity among the chlorosulfolipids because the configurations of **2** and **3** match those found in **6**. No characterization data besides mass spectrometric analyses for *O. danica* lipid **4** were available from the literature; therefore we have fully characterized a sample of desulfated **4** secured by one of us (T.H.). This disclosure provides complete characterization data for this

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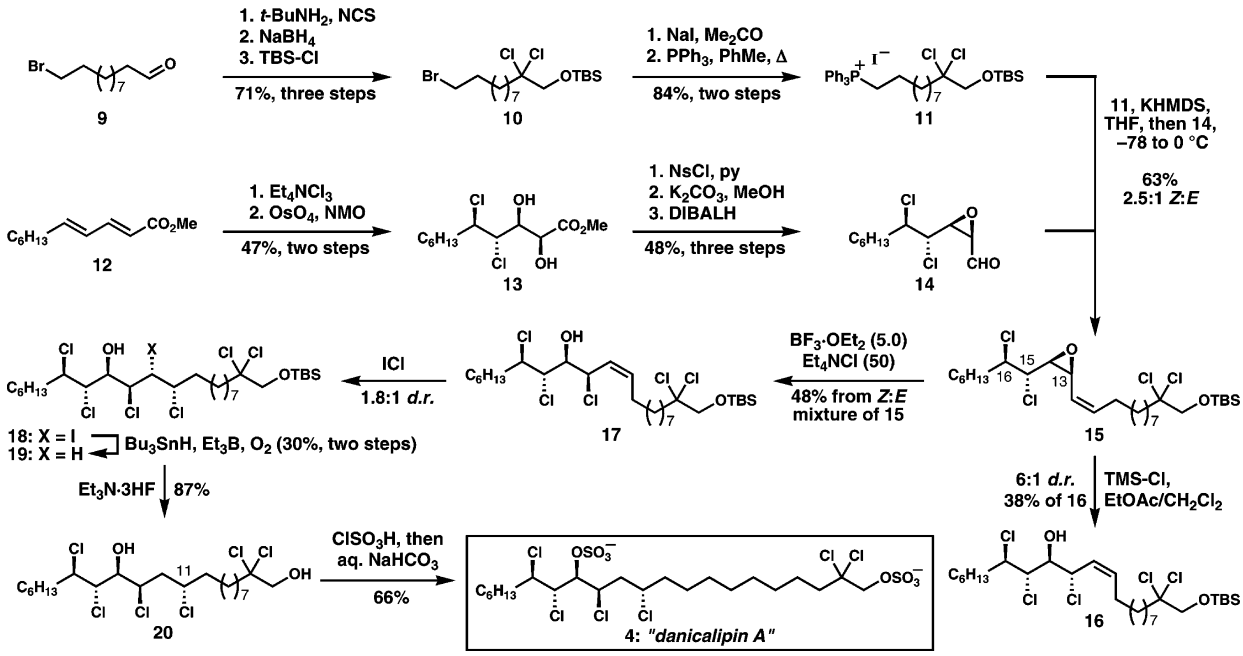
Scheme 1. Synthesis of "Danicalipin A", the Major Chlorosulfolipid from *Ochromonas danica*



diol,[10] the determination of its relative stereochemistry (shown in Scheme 1 as **20**) using Murata's *J*-based configurational analysis,[11] and the first laboratory synthesis of both the diol and lipid **4** according to a strategy that should also be applicable to chlorosulfolipids **5** and **6**.

Due to initial difficulties in implementing the *J*-based configurational analysis on the diol derived from lipid **4**, we proceeded on the assumption that stereochemistry would be conserved and that the relative stereochemistry of **4** would match that of **6**. After multiple unsuccessful attempts to build up the chlorine-rich motifs that characterize **4**−**6** using chloroacetate aldol or chloroallylation reactions with sensitive chloroaldehydes, we settled on a strategy that relied upon alkene functionalization reactions for introduction of the polar substituents.

Phosphonium salt **11** was prepared in five steps from 11-bromoundecanal **9**[12] according to the procedure shown in Scheme 1.[13] Anti-dichlorination of dienoic ester **12**[14] with Et_4NCl_3[15] and diastereoselective dihydroxylation[16] of the crude dichloride provided diol **13** in 47% overall yield. Epoxyaldehyde **14** was accessed via ring closure of the α-nosylate of **13** according to the procedure of Sharpless[17] followed by partial reduction of the ester.

Coupling of aldehyde **14** and salt **11** via Wittig reaction afforded vinyl epoxide **15** in 63% yield as a 2.5:1 mixture of alkenes favoring the desired *Z*-isomer. Vinyl epoxide opening with TMSCl according to the procedure of Llebaria[18] afforded mixtures of diastereomeric chlorohydrins that ranged from 6:1 to 1:1 *anti*/*syn* depending upon temperature; the *anti*-product **16** corresponds to a ring-opening process with net retention of C13-stereochemistry, explicable on the basis of anchimeric assistance by the C15- or C16-chloride, as described by Carreira.[9] While we were never able to reverse this ratio to favor the desired isomer using silyl chloride reagents, empirical studies led to the use of excess $BF_3{\cdot}OEt_2$ and Et_4NCl to afford the desired *syn*-chlorohydrin **17** in 48% yield directly from the *Z*/*E* isomeric mixture of **15**. Iodochlorination of the resulting *Z*-allylic chloride cleanly provided **18** with complete regiocontrol but poor stereoselectivity. The relative stereochemistry of the

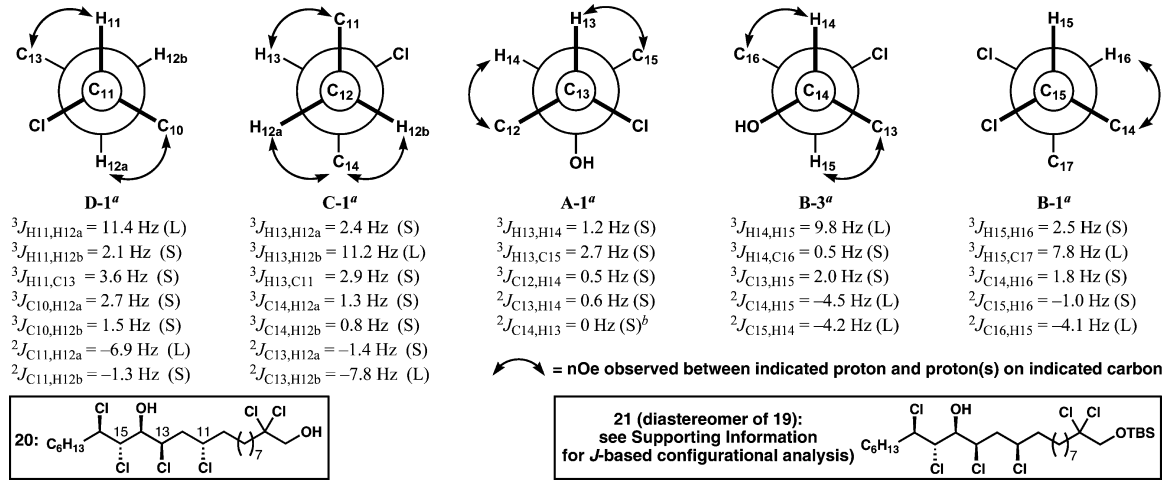


Figure 3. *J*-Based configurational analysis of diol **20** (synthetic material); long-range heteronuclear couplings obtained by HETLOC and HSQMBC experiments. The same analysis was performed with **21**; see Supporting Information for details. *a*. Relative configuration and rotamer designation following the convention of Murata et al.;[11] *b*. No correlation observed in HSQMBC as expected for a coupling of 0 Hz.

inseparable stereoisomers was not determined at this stage; however, after the crude mixture underwent selective reductive dehalogenation,[19] the resulting diastereomers could be painstakingly separated.[20] The low yield of the iodochlorination/deiodination sequence results from the difficulty of isolation of pure stereoisomer **19** and is not a consequence of poor reactivity; both steps are efficient based on analysis of crude reaction mixtures. Cleavage of the silyl ether of the major diastereomer of **19** afforded racemic diol **20**, for which spectral data were in complete agreement with those from the diol derived from natural sources. Only at this stage did the successful implementation of the *J*-based configurational analysis on **20** (see below) finally reveal the relative stereochemistry of this compound to be as shown in the scheme; unexpectedly, the configuration at C11 was opposite to that of the corresponding carbon in lipid **6**. Sulfation using typical reagents (SO_3·py, for example) provided NMR evidence for the formation of bis-sulfate **4**; however, isolation proved challenging because sulfate cleavage occurred readily upon aqueous workup or chromatography. The addition of $ClSO_3H$ to a $CDCl_3$ solution of **20** cleanly and rapidly provided racemic lipid in protonated form, which could be characterized but not isolated. Sequential treatment of **20** with excess $ClSO_3H$ in CH_2Cl_2 and saturated aqueous $NaHCO_3$ afforded **4** (bis Na salt), which was fully characterized for the first time (^1H and ^{13}C NMR, HRMS) and for which we offer the more tractable name danicalipin A.[21] To the best of our knowledge, none of the individual chlorosulfolipids of *O. danica* have previously been isolated in sulfated form.

The results of the *J*-based configurational analysis of **20** are shown in Figure 3. A combination of HETLOC and HSQMBC experiments[22] provided all of the requisite long-range heteronuclear coupling constants ($^{2,3}J_{C-H}$), and 1D-nOe experiments completed the analysis. Since application of this method to the chlorosulfolipids has only been validated once before, in Carreira's synthesis of **6**,[6a,9] caution was advisable; therefore, we also applied this analysis to the diastereomer of hexachloride **19** (see **21**, Figure 3). The results further supported our assignments.[10] Finally, the stereochemical outcome of the dichlorination/dihydroxylation sequence of **12** was secured by an X-ray crystallographic analysis on the α-nosylate derivative of **13**,[10] and a stereospecific epoxide formation reaction on **17** regenerated **15**,[10] proving that the **15** → **17** ring-opening transformation occurred with inversion of configuration.

While optimization of several steps is clearly warranted, this workable synthesis, in combination with the NMR studies described, establishes the relative stereochemistry of the major chlorosulfolipid from *O. danica* and demonstrates conservation of the relative stereochemistry in the C13−C16 region of this lipid with the corresponding segment of mussel-derived lipid **6**. The lack of conservation of stereochemistry at C11 is intriguing from a biosynthetic perspective and merits further investigation. The synthesis strategy described in this communication should be fully adaptable to the syntheses of other chlorosulfolipids such as **5** and **6** and should be suitable for the production of **4** in quantities sufficient to support studies of its role in membranes. Future chemical investigations include the development of enantioselective routes to **4** and the other chlorosulfolipids.

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Supporting Information Available: Experimental details, characterization data, stereochemical elucidation, and copies of NMR spectra for all new compounds are provided. A CIF file for a derivative of **13** is also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Full characterization of **21**, the C11-epimer of **19**, including *J*-based configurational analysis, further supports the stereochemical assignment of **19**, **20**, and **4**. See Supporting Information for details.
(21) The name danicalipin A was chosen based on its similarity to malhamensilipin A, which derives from *P. malhamensis*. Presumably, the other chlorosulfolipids of *O. danica* might be named danicalipins B, C, D, etc.
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