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Long-Chain 2*H*-Azirines with Heterogeneous Terminal Halogenation from the Marine Sponge *Dysidea fragilis*

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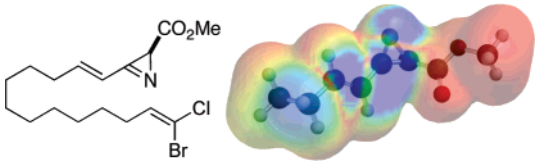

Three new ω-halogenated long-chain 2*H*-azirines were isolated from the sponge *Dysidea fragilis*. Their structures revealed heterogeneity in *both* the composition of the terminal 1,1-dihalo-vinyl group and enantiomeric ratios at C2 of the azirine-2-carboxylate ester terminus. Azirine-2-carboxylate esters were shown to racemize spontaneously. A hypothesis is proposed for the biosynthesis of the azirinecarboxylate family of natural products that involves enzyme-catalyzed free radical halogenation followed by elimination of hydrohalic acid.

Introduction

Halogenated natural products are abundant in Nature, with over 4500 unique compounds isolated and characterized from terrestrial and marine sources.[1] While the vast majority of these compounds (~98%) contain Br or Cl, few contain both. Polyketides and lipids (Figure 1) produced by polyketide synthases (PKS) and containing terminal halogenation are moderately rare, and their structures pose an intriguing biosynthetic question: how does Nature carry out terminal halogenation of ostensibly unactivated polyketide precursors?[2] Recently, Walsh and co-workers reported that oxidation of a threonine amino acid residue in syringomycin E is carried out by a novel α-ketoglutarate-dependent non-heme iron chlorinase that effects a "free radical" chlorine substitution.[3a] Analogous chlorination reactions in the cyanobacterium *Lyngbya majuscula*[3c] and sponge *Dysidea herbacea*[3d] produce trichloromethyl peptides (e.g., dysidenin and barbamide[3c]) by replacement of *all three* hydrogen atoms of the pro-4*S* methyl group in a leucine thioester residue. The latter halide substitutions occur at amino acid residues in peptide substrates of nonribosomal peptide synthases (NRPS) or PKS-NRPS clusters. The biosynthesis of terminal vinyl halides in PKS products, for example, in phorboxazole A,[4] the recently reported mutafuran G,[5] and spongistatin 1[6−8] (Figure 1, **i**, **iii**, and **iv**, respectively), is presently unknown.

We now report the isolation and complete stereostructures of three new halogenated long-chain lipids (**1**−**3**) from the marine sponge *Dysidea fragilis*, which present two unusual features: uncommon examples of 1-chloro-1-bromovinyl natural products (**1** and **2**, to the best of our knowledge, are the first examples of this functional group reported from marine invertebrates)[9] and heterogeneous enantiomeric composition of the even more rare azacyclopropene (2*H*-azirine) group in

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(1) (a) Gribble, G. W. *J. Chem. Ed.* **2004**, *81*, 1441−1449. (b) Gribble, G. W. *Acc. Chem. Res.* **1998**, *31*, 141−152. (c) Hartung, J. *Angew. Chem., Int. Ed.* **1999**, *38*, 1209−1211.

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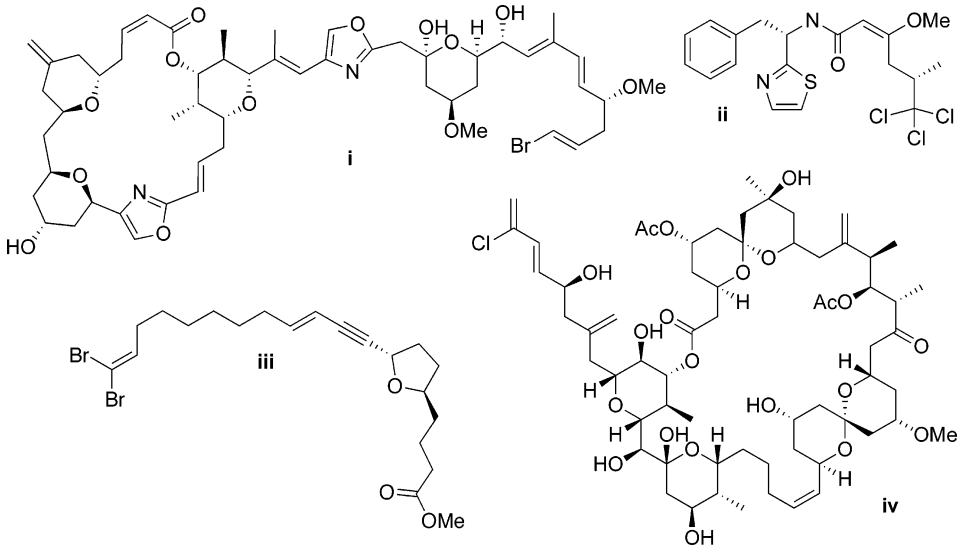

FIGURE 1. Halogenated marine natural products: **i**, phorboxazole A; **ii**, barbamide; **iii**, mutafuran G; **iv**, spongistatin 1.

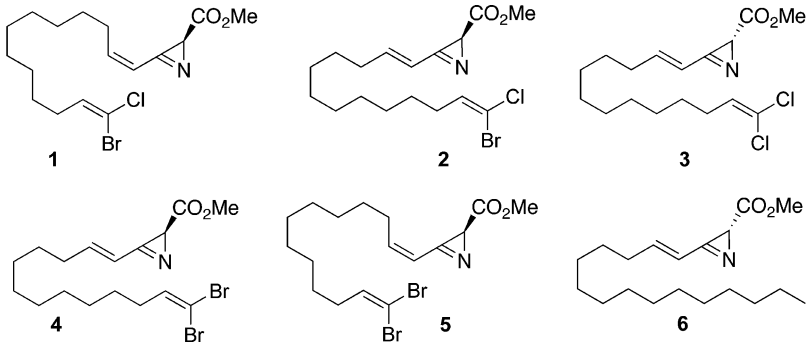

FIGURE 2. Structures of new compounds **1**−**3,** (+)-(*E*)-antazirine (**4**),[11] (+)-(*Z*)-antazirine (**5**),[11] and (−)-(*E*)-dysidazirine (**6**).[10]

compounds **1**−**3**. The structures shed light on end-group halogenation resulting in terminal vinyl halide natural products and possibly the origin of the 2*H*-azirine ring in Nature.

Results

Our chemical investigation of *D. fragilis* (collected in Pohnpei, 2001) was prompted by the high cytotoxicity of the crude extract (IC_{50} 0.17 μg/mL, HCT-116 cells) and observation of induction of early apoptosis in human tumor cell lines (Jurkat, HCT-116, PC-3, and MCF-7). The hexane-soluble fraction of the methanol extract of lyophilized *D. fragilis* contained long-chain azirines, related to dysidazirine,[10] that accounted for the activity. Pure compounds **1**−**5** were isolated by a combination of silica flash chromatography followed by HPLC on silica and reversed-phase silica to give the new azirines **1**−**3** along with the known natural products (*E*)-antazirine (**4**) and (*Z*)-antazirine (**5**).[11]

Comparison of the ^{1}H, ^{13}C NMR, and IR spectra of **1**−**3** with those of (*R*)-(−)-dysidazirine (**6**)[10] and antazirines (**4** and **5**)[11] showed almost identical features including a band in the FTIR spectrum at ν 1770 cm^{-1} due to the characteristic azirine C≡N stretch. However, small differences in the chemical shifts of the allylic carbon C15 suggested changes in substitution at the ω-terminus. Compounds **1** and **2** were isomeric with a formula of $C_{17}H_{25}BrClNO_2$ revealed by high-resolution EIMS and differed from the known azirines **4** and **5** by replacement of one Br for Cl. Compound **3**, $C_{17}H_{25}Cl_2NO_2$, has both Br's replaced by Cl. Therefore, compound **3** possessed a 1,1-dichloro-vinyl terminus, whereas a terminal 1-bromo-1-chlorovinyl group was confirmed for **1** and **2**.

Assignment of absolute configuration of **1**−**3** appeared to be a simple matter of comparison of optical rotations with that of (*R*)-(−)-**6**, the configuration of which was determined unambiguously by a combination of chemical degradation and circular dichroism.[10] To our surprise, each of **1**−**5** gave a different value for $[\alpha]_D$. Compound **3** was weakly levorotatory ($[\alpha]_D$ −4.1), whereas the **1**, **2** and **4**, **5** were dextrorotatory with larger magnitudes. Analysis of **1**−**5** by chiral HPLC (Figure 3 and Table 1) showed each was a mixture of enantiomers, with composition that varied from 78% ee to 4% ee. Interestingly, the % ee's of the (4*Z*)-isomers **1** and **5** are significantly higher than those of the corresponding (*E*)-isomers.

(9) The only other reported 1-bromo-1-chloro-1-alkenes from natural sources are simple aliphatic ketones, alcohols, and carboxylic acids from marine algae that may derive from haphazard electrophilic α-halogenation-elimination reactions of small molecular weight carbonyl compounds. (a) McConnell, O. J.; Fenical, W. *Tetrahedron Lett.* **1977**, *22*, 1851−1854. (b) McConnell, O. J.; Fenical, W. *Phytochemistry* **1980**, *19*, 233−247. (c) Woolard, F. X.; Moore, R. E. *Tetrahedron* **1976**, *32*, 2843−2846. (d) Burreson, B. J.; Moore, R. E.; Roller, P. P. *J. Agric. Food Chem.* **1976**, *24*, 856−861.

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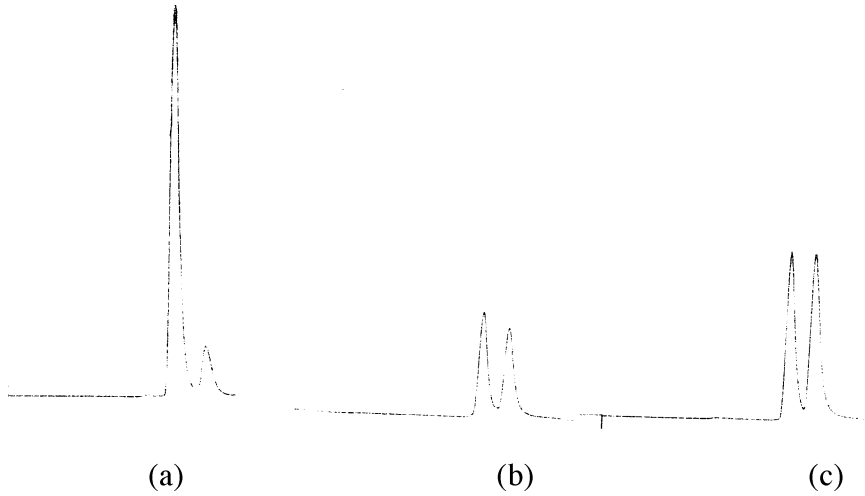


FIGURE 3. Chiral HPLC (Chiralpak AD, 85:15 hexanes/*i*-PrOH or 1:1 hexanes/*i*-PrOH, 0.5 mL/min) of **1** (a), **2** (b), and **3** (c).

TABLE 1. Enantiomeric Composition and Specific Rotations of Compounds 1−5

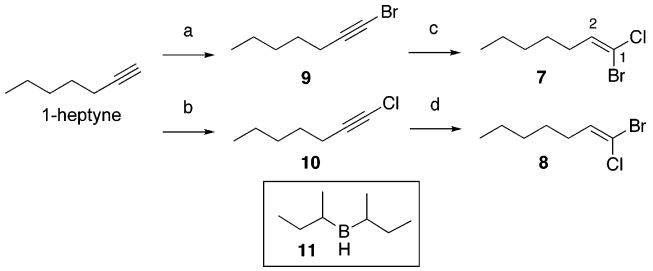
compd	abundance (%)[a]	% ee[b]	$[\alpha]_D$[c]	configuration
1	0.037	72	+96.9	2*S*,4*Z*,15*Z*
2	0.018	4	+8.9	2*S*,4*E*,15*Z*
3	0.020	9	−4.1	2*R*,4*E*
4	0.016	30	+16.7[d]	2*S*,4*E*
5	0.100	78	+98.9	2*S*,4*Z*
6	4.2	22	−33.5[e]	2*R*,4*E*

[a] Percent dry weight of sponge. [b] Determined by chiral HPLC (Chiralpak AD); see Figure 3. [c] Recorded in *n*-hexane at 24 °C. [d] Lit. +10.3.[11] [e] In MeOH, lit. −165°.[10] See also note in ref 20.

Faulkner first hypothesized that **4** and **5** may be nonracemic mixtures of enantiomers,[11] and Davis suggested that natural (−)-**6** may not be optically pure on the basis of comparison of $[\alpha]_D$ values of the synthetic and natural compounds.[12] To resolve this question, we also measured the enantiomeric composition of the original sample of natural (−)-**6**, originally isolated from *D. fragilis* collected in Fiji.[10] We found that this sample of **6**, dating back to 1988, was 22% ee, much lower than an original estimate based on a synthetic sample[12] and suggesting partial racemization had occurred.

Assignment of the terminal double bond geometry in **1** and **2** was a non-trivial problem. Stereospecific methods for synthesizing 1-bromo-1-chloro-1-alkenes have been reported,[13] but with insufficient spectroscopic data to allow comparison with **1**−**3**. NMR methods (e.g., NOE, calculation of ^{13}C NMR shifts) were sufficiently equivocal that we resorted to preparation of authentic *Z* and *E* model compounds for comparison of ^{13}C NMR chemical shifts with those of the natural products. We chose to synthesize (*Z*)- and (*E*)-1-chloro-1-bromo-1-heptene (**7** and **8**, respectively) based on the procedure of Masuda *et al.*[13a] (Scheme 1). Briefly, 1-bromoheptyne (**9**) and 1-chloroheptyne (**10**) were separately subjected to hydroboration with di-*sec*-butylborane followed by halogenation with $CuBr_2$ or $CuCl_2$ to give compounds **7** and **8**, respectively. The geometry of the resulting double bond in each compound is defined by anti-Markovnikov *syn* addition of the borane across the triple bond in the haloalkyne and retention of geometry during the subsequent boron displacement by halogen. Interestingly, no significant differences were observed in the ^1H NMR vinyl proton chemical shifts of **1**, **7**, or **8** (δ 6.07, t, $CDCl_3$); however the ^{13}C NMR chemical shift of the sp^2 quaternary carbon was sensitive to the geometry of the olefin (**1**, δ 106.2, s; (*Z*)-**7**, 106.2, s; (*E*)-**8**, 103.7, s, $CDCl_3$). Hence the geometry of the terminal olefin in **1** and **2** can be assigned as *Z*.

SCHEME 1. Synthesis of Model Compounds 7 and 8[a]



[a] Reagents and conditions: (a) $AgNO_3$, NBS, acetone, 23 °C, 2 h, 81%; (b) (i) *n*-BuLi, THF, −25 °C, 1 h, (ii) NCS, THF, 2.25 h, − 25 °C to rt, 58% over two steps; (c) (i) **11**, THF, −15 °C to rt, 3 h, (ii) $CuCl_2$, DMA, THF/H_2O, 0 °C to rt, 16 h, 5% over 2 steps; (d) (i) **11**, THF, −15 °C to rt, 3 h, (ii) $CuBr_2$, DMA, THF/H_2O, 0 °C to rt, 16 h, 10% over two steps.

TABLE 2. In Vitro Cytotoxicity Data of 1−5 against HCT-116 Cells[a]

compound	IC_{50} (μg/mL)	IC_{50} (μM)
1	5.3	13.6
2	5.9	15.2
3	8.6	24.8
4	8.5	19.6
5	7.9	18.2

[a] Cells were grown for 20 h (37 °C, 5% CO_2), treated with drug, and then grown for 3.5 days. Cell viability was measured by the MTS endpoint (soluble formazan dye); MTS = (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt.

Compounds **1**−**5** displayed moderate *in vitro* cytotoxic activity against HCT-116 cells (Table 2). Although (*R*)-(−)-dysidazirine (**6**) was reported to show significant in vitro activity against the pathogenic yeast *Candida albicans*,[10] we found **1**−**5** were inactive against the same organism and seven other yeasts (*C. albicans* ATCC 14503, *C. albicans* UCD-FR1, *C.*

(12) Davis, F. A.; Reddy, G. V.; Liu, H. *J. Am. Chem. Soc.* **1995**, *117*, 3651−3652.

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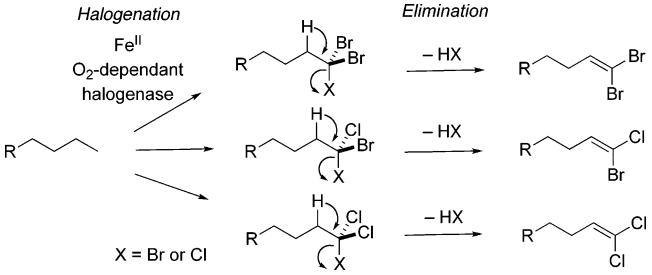
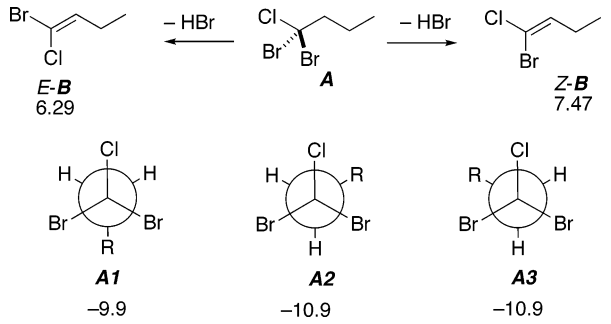
SCHEME 2. Possible Biosynthesis of 1,1-Dihalogenated-1-alkenes



SCHEME 3. Calculated Energies of 1-Chloro-1,1-dibromobutane Conformers A1−3 and Elimination Products *E*-B and *Z*-B (kcal mol^{-1}, MMFF, Spartan 04)



albicans 96-489, *C. glabrata*, *C. krusei*, *Cryptococcus neoformans* var. *grubii*, and *Cryptococcus neoformans* var. *gatti*).[14] Apparently chain length, end-group substitutions, or both are important in modulating *in vitro* antifungal activity of long-chain 2*H*-azirines.

Discussion

Compounds **1**−**5** comprise an unusually high percentage of the hexane-soluble lipid fraction (15.5% w/w) of *D. fragilis*. Two suggestions have been proposed for biosynthesis of azirines: an aberrant shunt reaction of sphingolipid biosynthesis[10] or "a possible biosynthetic scheme involving formation of the azirine ring by a mechanism involving bromination/dehydrobromination" as intimated by Faulkner.[11] The origin of ω-vinyl halides in long-chain lipids is also unknown; however, a common theme of halogenation at *each* of the termini may unify the biogenesis of **1**−**5**: free-radical halogenation of an extended starter unit in the growing polyketide chain followed by a *single* β-elimination (Scheme 2) and a different *double* halogenation-dehydrohalogenation at the α-terminus (Scheme 4).

It is interesting to note that the terminal 1,1-dihalovinyl groups in compounds **1**−**5** represent all possible permutations that could arise from promiscuous triple free radical halogenation by Br or Cl followed by a base-induced β-elimination of HX (Scheme 2), except the (15*E*)-isomers of **1** and **2**.[15] The (15*Z*)-alkene, although calculated to be less stable than the "missing" (15*E*)-isomer by approximately 1.3 kcal mol^{-1} (Spartan '04, PM3 level) would be the favored *kinetic* product of *E*2 elimination of HBr from the corresponding 1,1-dibromo-1-chloroalkane by virtue of entropic factors that are best illustrated by the following example of a simple model. Of the three staggered conformations of 1-chloro-1,1-dibromobutane (**A1** and the two degenerate conformers, **A2** and **A3**, Scheme 3), *anti*-periplanar *E*2 elimination of HBr from **A2**/**A3** is expected to give rise to the (*E*)-geometrical isomer of 1-bromo-1-chloro-1-butene. On the other hand, **A1**, which lies only 1.2 kcal mol^{-1} higher in energy than **A2**/**A3**, provides two favorable *E*2 pathways for elimination of HBr, each leading to the (*Z*)-isomer exclusively.

While the isolation of all five compounds from the same specimen of *D. fragilis* supports terminal radical halogenation in **1**−**5**, there are also recent precedents for this hypothesis. Walsh et al. demonstrated[3b] that two Fe^{II}, O_2-dependent halogenases, encoded by the *BarB1*/*BarB2* synthase gene cluster,[3c] are responsible for iterative triple chlorination of the terminal *pro-S*-methyl group of L-leucine in the biosynthesis of barbamide (Figure 1, **ii**).[3c] The homologous enzyme *SyrB2* from *Pseudomonas syringae* pv. *syringae* B301D installs chlorine at C4 of the threonine (Thr) residue in syringomycin E.[3a] SyrB2 is somewhat promiscuous; it can utilize Br^- in place of Cl^- to convert Thr to 4-bromo-L-Thr, albeit with a lower selectivity (∼180:1 favoring Cl^-).[16] Indeed, dechloro-bromosyringomycin E was produced when *Pseudomonas syringae* was cultured in the presence of excess NaBr.[17] Finally, the biosynthesis of the cyclopropyl amino acid coronamic acid (CMA, Scheme 4a) proceeds through "cryptic halogenation"; chlorination of the γ-methyl group of L-*allo*-isoleucine is followed by a base-induced cyclization that breaks the nascent carbon-chlorine bond by intramolecular S_N2 displacement by the α-enolate to form the cyclopropane ring.[18] Although speculation for the biosynthesis of a trichloromethyl group by sequential triple halogenation of leucine dates back to 1977 with the isolation of the marine natural product dysidenin[21a] and experimental evidence for ^{14}C-leucine incorporation into demethyldysidenin by the sponge *Dysidea herbacea* was communicated in 1992,[21b] the putative intermediacy of heterogeneously substituted bromodichloromethyl groups and tribromomethyl groups (Schemes 2 and 3) is unprecedented and deserving of further investigation.

The mixture of enantiomers observed for **1**−**5** requires no special explanation since the pK_a of H2 is expected to be appreciable and the compounds racemize slowly under auto-

(14) *Candida albicans* ATCC 14503 and UCD-FR1 (selected by passage through Fluconazole-containing media, are resistant to Fluconazole (100 μg/mL). Clinical isolates of *Candida glabrata* were provided by Kathy Holton (University of Texas Medical Center), and *Cryptococcus neoformans* var. *grubii* and *gatti* were provided by Prof. Angie Gelli (University of California, Davis School of Medicine).

(15) We have not been able to detect the presence of (15*E*)-isomer of **1** or **2** in the azirine-containing fraction of *D. fragilis* (silica HPLC, UV λ 254 nm, upper limit of detection ∼0.007% dry weight of sponge).

(16) Vaillancourt, F. H.; Vosburg, D. A.; Walsh, C. T. *ChemBioChem* **2006**, *7*, 748−752.

(17) Grgurina, I.: Barca, A.; Cervigni, S.; Gallo, M.; Scaloni, A.; Pucci, P. *Experientia* **1994**, *50*, 130−133.

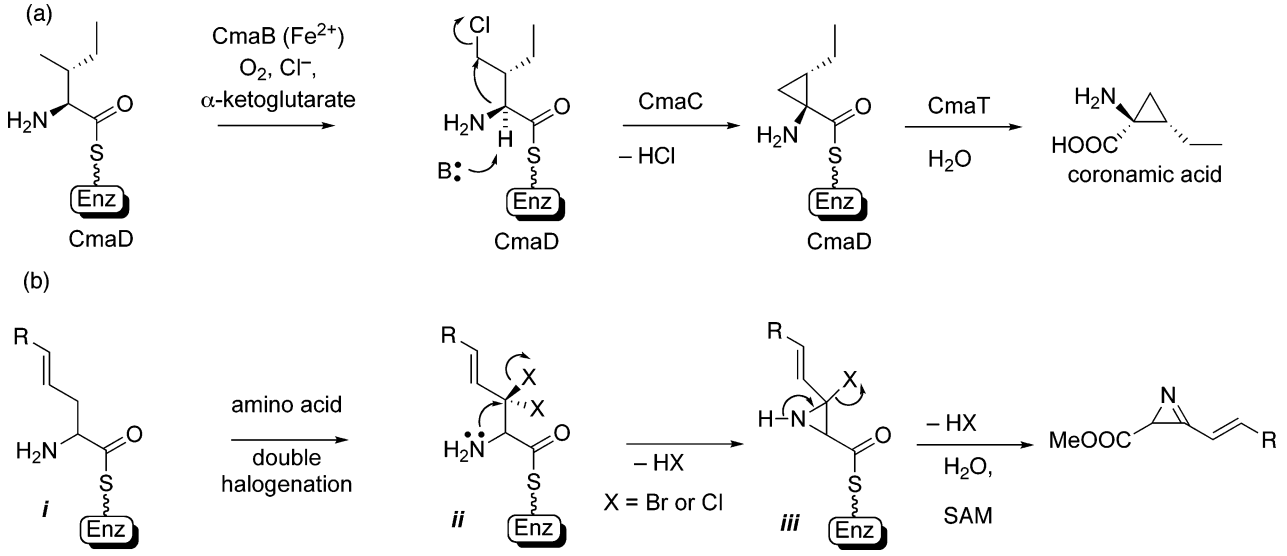
(18) Vaillancourt, F. H.; Yeh, E.; Vosburg, D. A.; O'Connor, S. E.; Walsh, C. T. *Nature* **2005**, *436*, 1191−1194.

(19) (a) Emmert, E. A.; Klimowicz, A. K.; Thomas, M. G.; Handelsman, J. *Appl. Environ. Microbiol.* **2004**, *70*, 104. (b) Stohl, E. A.; Milner, J. L.; Handelsman, J. *Gene* **1999**, *237*, 403.

(20) The calculated pK_a (HA$^+$) of azirine is 4.56 (CAS reg no. 157-16-4, ACD/Labs software) or slightly less basic than pyridine. The C2 enolates of **1**−**5** should be stabilized by delocalization of the negative charge. Azirines **1**−**6** were chemically stable when stored at −20 °C as free bases. Remarkably, the original sample of (*R*)-(−)-dysidazirine (**6**) from Fiji[10] was found to be unchanged after 19 years in storage at −20 °C (^{1}H NMR, FTIR) except for the % ee, which eroded from 89% (estimated[12] from the measured $[\alpha]_D$ −165° in ∼November 1987[10]) to 22% (chiral HPLC) and $[\alpha]_D$ −33.5° (August 2007, see Supporting Information). Assuming an exponential first order rate law for racemization, we calculated the half-life of **6**, $t_{1/2}$ = 9.4 years at −20 °C.

(21) (a) Kazlauskas, R.; Lidgard, R. O.; Wells, R. J.; Vetter, W. *Tetrahedron Lett.* **1977**, 3183−3186. (b) Garson, M. J. In *Sponges in Space and Time: Biology, Chemistry and Pharmacology*; A. A. Balkema: Amsterdam, 1994; p 437.

SCHEME 4. (a) Cryptic Halogenation in the Biosynthesis of Coronamic Acid;[18] (b) Proposal for the Biosynthesis of 2-Carboxy-2*H*-azirines



catalysis.[20] It is interesting to note that **1** and **5**, which possess a (*Z*) olefin at C4−C5, occur with significantly higher % ee's than **2**−**4**, which possess the (*E*) double bond. As no optical rotation was reported for **5** in the original isolation,[11] it remains to be seen if this trend is general for antazirines or simply a coincidence for this sample. The origin of the nitrogen atom poses an interesting question. Whether the 2*H*-azirine ring arises from incorporation of an unusual nitrogenous terminal ketide starter unit (cf. 2-aminomalonyl CoA incorporation in zwittermicin A[19]) or from deviated sphingolipid biosynthesis, the nitrogen will be traceable to L-serine in both cases. Single ^{15}N-serine labeling experiments may not discriminate between these two possible pathways; however, triple labeling with [1,2-$^{13}C_2$,^{15}N]-serine may.

Conclusion

In summary, three new long-chain 2*H*-azirines (**1**−**3**) have been isolated from the marine sponge *D. fragilis*, two containing a terminal (*Z*)-1-bromo-1-chlorovinyl group, the first such example from a marine invertebrate. The heterogeneity of terminal halogenation and enantiomeric composition in **1**−**3** and other known long-chain azirines suggest a common halogenation motif in the biosynthesis of the two chain termini.

Experimental Section

Animal Material. *Dysidea fragilis* (01-18-154) was collected using scuba at Arrow Wall, Pohnpei, Federated States of Micronesia in September, 2001. The sample was frozen (652.3 g wet weight) and stored at −20 °C until extraction. A voucher sample of the sponge is kept at UC San Diego.

Extraction and Isolation. Freeze-dried sponge (182.5 g dry weight) was soaked in MeOH (1.2 L) for 4 h. The methanol extract was filtered off, fresh MeOH (1.2 L) was added, and the sponge was allowed to soak overnight. The combined MeOH extracts were concentrated under reduced pressure to ∼500 mL. Water (75 mL) was added, and the aqueous mixture was partitioned against hexanes (500 mL). The aqueous MeOH layer was separated and diluted with a further 75 mL of water and then partitioned against $CHCl_3$ (500 mL). After separation the aqueous MeOH layer was diluted with water (100 mL) and re-extracted with $CHCl_3$. *n*-BuOH (20 mL) was added to the aqueous MeOH layer, and the MeOH was removed under reduced pressure. The remaining aqueous layer was partitioned twice against *n*-butanol (250 mL).

The hexanes-soluble fraction was evaporated to dryness, giving a dark oil (2.24 g). A portion of this fraction (1.67 g) was subjected to gradient flash chromatography (10−100% EtOAc/hexanes). The second collected fraction eluting at 1:9 EtOAc/hexanes (468 mg, orange oil) was purified by passage through a reversed phase cartridge (C_{18}, 20 g, 5:95 H_2O/MeOH) to give a mixture of compounds **1**−**3** and (*E*)-antazirine (**4**) and (*Z*)-antazirine (**5**).[11] This mixture was subjected to silica HPLC (Dynamax Microsorb, 1:19 EtOAc/hexanes, 15 mL/min, λ 254 nm), yielding three fractions. Reversed phase HPLC separation (Dynamax Microsorb C18, 17.5:82.5 H_2O/MeOH, 15 mL/min, λ 254 nm) of the three fractions yielded **4** (22 mg, 0.016%), **5** (136 mg, 0.10%), **1** (50 mg, 0.037%), **2** (24 mg, 0.018%), and **3** (27 mg, 0.02%), each as a clear, colorless oil.

(4*Z*,15*Z*)-(*S*)-1. $[\alpha]^{24}$ +96.9° (*c* 1.09, *n*-hexane); UV (*n*-hexane) 210 nm (ϵ 11 767); IR (thin film) ν_{max} 2925, 2855, 1759, 1732, 1611, 1433, 1336, 1200, 1180, 1025, 846, 799 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.55 (dt, 1H, J = 10.5, 8.0 Hz, H5), 6.41 (dt, 1H, J = 10.5, 1.2 Hz, H4) 6.06 (t, 1H, J = 7.5 Hz, H15), 3.71 (s, 3H, OMe), 2.61 (s, 1H, H2), 2.48 (m, 2H, H6), 2.09 (q, 2H, J = 7.5 Hz, H14), 1.43−1.35 (br m, 2H), 1.25 (br s, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0 (C, C1), 154.0 (C, C3), 152.4 (CH, C5), 133.8 (CH, C15), 111.1 (CH, C4), 106.2 (C, C16), 52.3 (CH_3, OMe), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.38 (CH_2), 29.35 (CH_2), 29.32 (CH, C2), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.0 (CH_2); LRESIMS *m*/*z* 392.04 $[M + H]^+$; HREIMS *m*/*z* 389.0754 (calcd for $C_{17}H_{25}BrClNO_2$, 389.0752).

(4*E*,15*Z*)-(*S*)-2. $[\alpha]^{24}$ +8.9° (*c* 0.51, *n*-hexane); UV (*n*-hexane) 210 nm (ϵ 15 660); IR (thin film) ν_{max} 2925, 2846, 1767, 1728, 1468, 1433, 1336, 1262, 1200, 1184 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (dt, 1H, J = 15.4, 6.8 Hz, H5), 6.53 (dt, 1H, J = 15.4, 1.4 Hz, H4), 6.06 (t, 1H, J = 7.3 Hz, H15), 3.71 (s, 3H, OMe), 2.56 (s, 1H, H2), 2.35 (br q, 2H, J = 6.8 Hz, H6), 2.10 (q, 2H, J = 7.3 Hz, H14), 1.53−1.46 (br m, 2H), 1.42−1.35 (br m, 2H), 1.26 (br s, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1 (C, C1), 156.6 (C, C3), 155.6 (CH, C5), 133.9 (CH, C15), 112.9 (CH, C4), 106.3 (C, C16), 52.2 (CH_3, OMe), 33.2 (CH_2), 31.8 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.3 (CH, C2), 27.9 (CH_2), 27.8 (CH_2); LRESIMS *m*/*z* 392.01 $[M + H]^+$; HREIMS *m*/*z* 389.0755 (calcd for $C_{17}H_{25}BrClNO_2$, 389.0752).

(4*E*)-(*R*)-3. $[\alpha]^{24}$ −4.1° (*c* 0.62, *n*-hexane); UV (*n*-hexane) 218 nm (ϵ 14 235); IR (thin film) ν_{max} 2925, 2846, 1774, 1735, 1623,

1434, 1341, 1264, 1200, 1035, 972, 875, 793, 725 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.68 (dt, 1H, $J = 15.3$, 7.0 Hz, H5), 6.53 (dt, 1H, $J = 15.3$, 1.5 Hz, H4), 5.83 (t, 1H, $J = 7.4$ Hz, H15), 3.71 (s, 3H, OMe), 2.56 (s, 1H, H2), 2.35 (br q, 2H, $J = 7.0$ Hz, H6), 2.14 (q, 2H, $J = 7.4$ Hz, H14), 1.52−1.46 (br m, 2H), 1.41−1.35 (br m, 2H), 1.27 (br s, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2 (C, C1), 156.6 (C, C3), 155.7 (CH, C5), 130.0 (CH, C15), 119.7 (C, C16), 112.9 (CH, C4), 52.2 (CH_3, OMe), 33.2 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.24 (CH_2), 29.22 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.3 (CH, C2), 28.1 (CH_2), 27.8 (CH_2); HREIMS *m*/*z* 345.1255 (calcd for $C_{17}H_{25}Cl_2NO_2$, 345.1257).

(+)-(*E*)-(*S*)-Antazirine (4).[11] $[\alpha]^{24}$ +16.7° (*c* 0.58, *n*-hexane), lit. +10.3° (*c* 0.39, $CHCl_3$);[11] LRESIMS *m*/*z* 435.98 $[M + H]^+$. See Table 1 for enantiomeric composition.

(+)-(*Z*)-(*S*)-Antazirine (5).[11] $[\alpha]^{24}$ +98.9° (*c* 3.33, *n*-hexane); LRESIMS *m*/*z* 435.99 $[M + H]^+$. See Table 1 for enantiomeric composition.

Preparation of 1-Bromo-1-chloro Heptenes. Compounds **7** and **8** were prepared according to the method of Masuda et al.[13a] 1-Heptyne was halogenated (NBS/$AgNO_3$) to give 1-bromoheptyne (**9**) or treated with *n*-BuLi/NCS to give 1-chloroheptyne (**10**). Alkynes **9** and **10** were then subjected to hydroboration with di-*sec*-butylborane followed by halogenation with either CuCl or $CuBr_2$ to give **7** and **8**, respectively. The products were separately purified after extractive workup with pentane by HPLC (silica, pentane, 3 mL/min, followed by C_{18} reversed phase HPLC, MeOH, 3.5 mL/min, refractive index detection).

(*Z*)-1-Bromo-1-chloro-1-heptene (7). IR (neat) ν 2917, 2851, 1476, 1464, 866, 726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.07 (t, 1H, 7.2 Hz), 2.10 (q, 2H, 7.2 Hz), 1.40 (m, 2H), 1.34−1.25 (m, 4H), 0.88 (t, 3H, 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.0 (CH), 106.2 (C), 31.9 (CH_2), 31.2 (CH_2), 27.7 (CH_2), 22.4 (CH_2), 14.0 (CH_3); HREIMS *m*/*z* 209.9808 (calcd for $C_7H_{12}BrCl$, 209.9805).

(*E*)-1-Bromo-1-chloro-1-heptene (8). IR (neat) ν 2958, 2933, 2859, 1608, 1456, 827 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.07 (t, 1H, 7.4 Hz), 2.12 (q, 2H, 7.4 Hz), 1.39 (m, 2H), 1.35−1.24 (m, 4H), 0.88 (t, 3H, 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.2 (CH), 103.7 (C), 31.2 (CH_2), 30.5 (CH_2), 27.7 (CH_2), 22.4 (CH_2), 14.0 (CH_3); HREIMS *m*/*z* 209.9805 (calcd for $C_7H_{12}BrCl$, 209.9805).

Chiral HPLC Analysis of Natural (*R*)-(*E*)-Dysidazirine (6). The original purified sample of natural dysidazirine (**6**, isolated from *D. fragilis*, collected in Fiji, 1987[10] and stored at −20 °C since 1988) was verified as unchanged by ^1H NMR (500 MHz, $CDCl_3$). The sample was analyzed by chiral HPLC (Chiralpak AD, 1:9 *i*-PrOH/hexanes, 0.5 mL/min). Integration of peaks corresponding to *S* and *R* enantiomers indicated 22% ee: $[\alpha]^{22}$ −33.5° (*c* 1.29, MeOH) (lit.[10] $[\alpha]_D$ −165° (*c* 0.5, MeOH)). For HPLC comparison, an authentic sample of synthetic (2*R*)-(*E*)-dysidazirine (53% ee) was prepared by photochemical isomerization (sunlamp, catalytic I_2, CH_2Cl_2) of synthetic (*R*)-(*Z*)-dysidazirine, (53% ee)[22] and analyzed under the same conditions (co-injection) (see Supporting Information for chromatograms and the exponential fit for first-order racemization).

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Supporting Information Available: Chiral HPLC chromatograms of aged **6**, exponential fit for first-order racemization of **6**, and ^1H and ^{13}C NMR spectra of **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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