Supporting Information

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SI Methods

General. Semipreparative high performance liquid chromatography (HPLC) was performed using a Waters 1525 or 515 pump with a Waters 2996 diode-array UV detector or a Waters 2487 dual-wavelength absorbance detector. Compound purification was achieved with Agilent Zorbax SB-C18 and RX-SIL columns (5 μm, 9.4 $\times$ 250 mm). ^{1}H NMR spectra were collected in $CDCl_3$ on a Bruker DRX-500 instrument with a 5-mm broadband probe and referenced to residual $CHCl_3$ (7.24 ppm). LC-MS analyses were conducted with a Waters 2695 HPLC interfaced to a 2996 diode-array UV detector and Micromass ZQ 2000 electrospray ionization mass spectrometer, using MassLynx 4.0 software and an Alltech Alltima C_{18} reversed-phase column (3 μm, 2.1 $\times$ 150 mm). HPLC grade solvents were used in semipreparative HPLC and DESI-MS, and optima grade solvents applied in LC-MS experiments (Fisher Scientific). NMR solvents were obtained from Cambridge Isotope Laboratories. High resolution mass spectra were acquired using electrospray ionization with an Applied Biosystems QSTAR-XL hybrid quadrupole-time-of-flight tandem mass spectrometer and Analyst QS software. Epifluorescence and light microscopy experiments were conducted with an Olympus IX50 inverted microscope, and images collected with MagnaFire software (Optronics). Additional light micrographs were obtained with an Olympus dissecting scope (i.e., Fig. S5). All statistical analyses were completed with either SYSTAT version 9 or GraphPad version 4.

The Marine Alga *Callophycus serratus*. The red macroalga *C. serratus* (Harvey ex Kutzing 1957) (family Solieriaceae, order Gigartinales, class Rhodophyceae, phylum Rhodophyta) was collected at depths from 1 to 30 m at several sites in Fiji. Ten collections were made in 2006 at Yanuca, Waitabu in Taveuni, Lavena in Taveuni, and Dravuni in Kadavu; GPS coordinates for each collection are provided in Table S1. Immediately following collection, portions for quantitative whole tissue LC-MS experiments were extracted as described in the main text. Remaining material was frozen at −20 °C until further processing. Samples for DESI-MS experiments and microscopic analyses were collected in 2008 at Yanuca (18°22′35″ S, 177°59′72″ E) and immediately preserved with 1% or 10% formalin in natural seawater until analysis. Samples for DESI-MS analyses were from separate plants collected on the reef at distances from 3 to 1,000 m. Algal samples were identified based on comparison with previously described morphological traits (S. Fredericq, personal communication) and by 18S rRNA sequencing (see below). Vouchers are housed at the Georgia Institute of Technology and the University of the South Pacific in Suva, Fiji.

18S rRNA Sequencing. Genomic DNA from ethanol-preserved *Callophycus serratus* samples (G0004, G0021, G0039, G0049, G0052, G0100, G0113, G0118 and G0171) was extracted using the DNeasy Tissue Extraction Kit (Qiagen) and purified using polyethylene glycol. The nuclear small subunit ribosomal RNA (18S rRNA) gene was amplified via PCR, in 3 separate reactions, using primers (G01/G09, G02/G08, and G04/G07) and reaction conditions from Saunders and Kraft (1). Each of the 3 18S rRNA fragments was sequenced in both directions. Sequences were manually edited in BioEdit vers 7.0.5.3 (2) and aligned using ClustalW (3). Sequences were deposited in GenBank (accessions FJ660605-FJ660613). Phylogenetic relationships among sequences were determined in PAUP* (4), using parsimony criteria and 1,000 bootstrap replicates, including *Callophycus oppositifolius* as an outgroup (GenBank accession no. AY437654)

Antimicrobial Assays. Assays with *Lindra thalassiae* (ATCC 56663) were completed as described in ref. 5. HP20ss fractions were solubilized in a minimal volume of acetone and incorporated into molten YPM agar (16 g/L granulated agar, 2 g/L yeast extract, 2 g/L peptone, 4 g/L D-mannitol, 250 mg/L of both streptomycin sulfate and penicillin G in 1 L of natural seawater) at concentrations approximating natural whole algal tissue concentrations. For each fraction, three 400-μL subsamples of this mixture were dispensed into sterile 24-well microtiter plates, allowed to solidify, and an aliquot of *L. thalassiae* suspension in sterile seawater added to each well. Control wells were prepared with YPM agar and acetone but no algal material. Plates were incubated at 28 °C for 3 days and digital photographs collected for each well. The area calculator feature of ImageJ software (National Institutes of Health) was applied to determine the percentage of each well covered in fungal hyphae, and for each chromatographic fraction, the average fungal coverage for the 3 subsamples was treated as a single replicate. Assays were conducted for the bromophycolide- or callophycoic acid/callophycol-containing fractions from $n = 4$ independent bromophycolide chemotype *C. serratus* collections and $n = 6$ callophycoic acid/callophycol chemotype collections. Fungal coverage for treatments vs. controls and among different treatments was compared using 1-way ANOVA with Dunnett's post test (6).

Antifungal assays with pure bromophycolides, callophycoic acids, and callophycols were completed as with extract fractions. Individual compounds were incorporated into molten YPM agar at 1:1 serially diluted concentrations ranging from 300 μM to 0.15 μM and $n = 3$ assays completed at each concentration. Significant growth inhibition at the maximum tested concentration was established by comparison of individual treatments with solvent-only controls applying 1-way ANOVA with Dunnett's post test (6). For compounds significantly inhibitory ($P \leq 0.05$) at the maximum tested concentration, inhibition data were fit to a sigmoidal dose-response curve; mean log IC_{50} and standard error values were calculated. Reported IC_{50} values were determined by computing the antilog of mean log IC_{50} values; standard errors for IC_{50} values were not determined, because such values are not directly correlated with log IC_{50} standard errors (7). Significant antifungal activity differences among active compounds were analyzed with an F test of the log IC_{50} value for each compound (7).

Antibacterial assays using *Pseudoalteromonas bacteriolytica* (ATCC 700679) were adapted from reported methods (5). A 24-h shake culture of *P. bacteriolytica* was diluted 1:160 in Difco Marine Broth 2216 (BD Biosciences) and 195 μL of this mixture added to duplicate treatment and control wells of a 96-well plate. An equal amount of sterile marine broth was added to blank wells. Five microliters of 40$\times$ concentrated HP20ss fractions in DMSO were then dispensed into all treatment and blank wells, giving a final concentration approximating natural whole tissue concentrations in the alga; 5 μL of DMSO were added to corresponding control wells. Plates were incubated at 30 °C for 24 h and turbidity measured at a wavelength of 600 nm. We corrected for the natural absorbance of extract fractions by subtracting extract-only sterile blank turbidities from values obtained for treatments. For chromatographic fractions from each *C. serratus* collection, the average turbidity value from 2

subsample assays was treated as a single replicate. Assays were conducted for the bromophycolide- or callophycoic acid/callophycol-containing fractions from $n = 4$ independent bromophycolide chemotype *C. serratus* collections and $n = 6$ callophycoic acid/callophycol chemotype collections. Turbidities were statically compared with 1 those obtained for no-extract controls, using 1-tailed paired t test (6).

To establish a role of bromophycolides in antifungal defense of algal surfaces, 2:1 bromophycolide A and B solutions were serially diluted (1:1) in ethyl ether and 30-μL aliquots dispensed as evenly as possible over the surface of 400 μL of solidified YPM agar blocks (200-mm^2 area) in 24-well microtiter plates, and allowed to air dry. Assuming even distribution of bromophycolides A and B across agar surfaces and negligible absorption of compounds into agar blocks, this corresponded to combined surface concentrations ranging from 340 pmol/mm^2 to 1.3 pmol/mm^2 ($n = 2$ assays at each concentration). Solvent-only control wells were prepared equivalently. Surfaces of treatment and control wells were inoculated with a suspension of *L. thalassiae* in sterile seawater and incubated at 28 °C for 3 days. *L. thalassiae* growth was assessed and the log IC_{50} value computed as before.

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1. Saunders GW, Kraft GT (1994) Small-subunit rRNA gene sequences from representatives of selected families of the Gigartinales and Rhodymeniales (Rhodophyta). 1. Evidence for the Plocamiales ord. nov. *Can J Bot* 72:1252–1263.
2. Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98.
3. Larkin MA, et al. (2007) ClustalW and ClustalX version 2. *Bioinformatics* 23:2947–2948.
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7. Motulsky HJ and Christopoulos A (2003) *Fitting Models to Biological Data Using Linear and Nonlinear Regression. A Practical Guide to Curve Fitting* (GraphPad Software, San Diego).

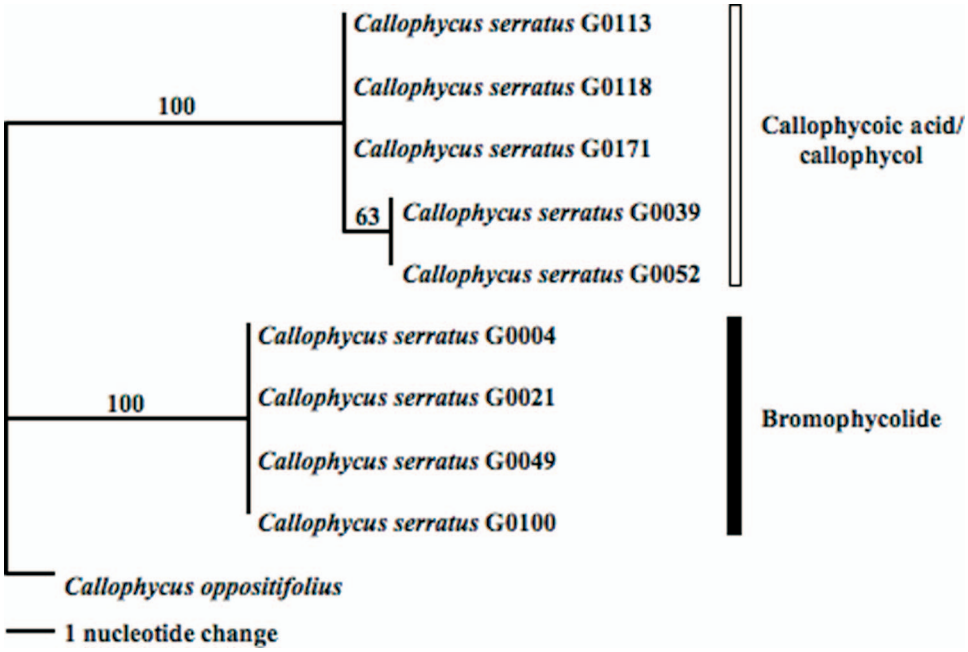

Fig. S1. Phylogenetic analysis of *Callophycus serratus* 18S rRNA sequences with corresponding bromophycolide or callophycoic acid/callophycol chemotype indicated. Numbers on branches indicate bootstrap support.

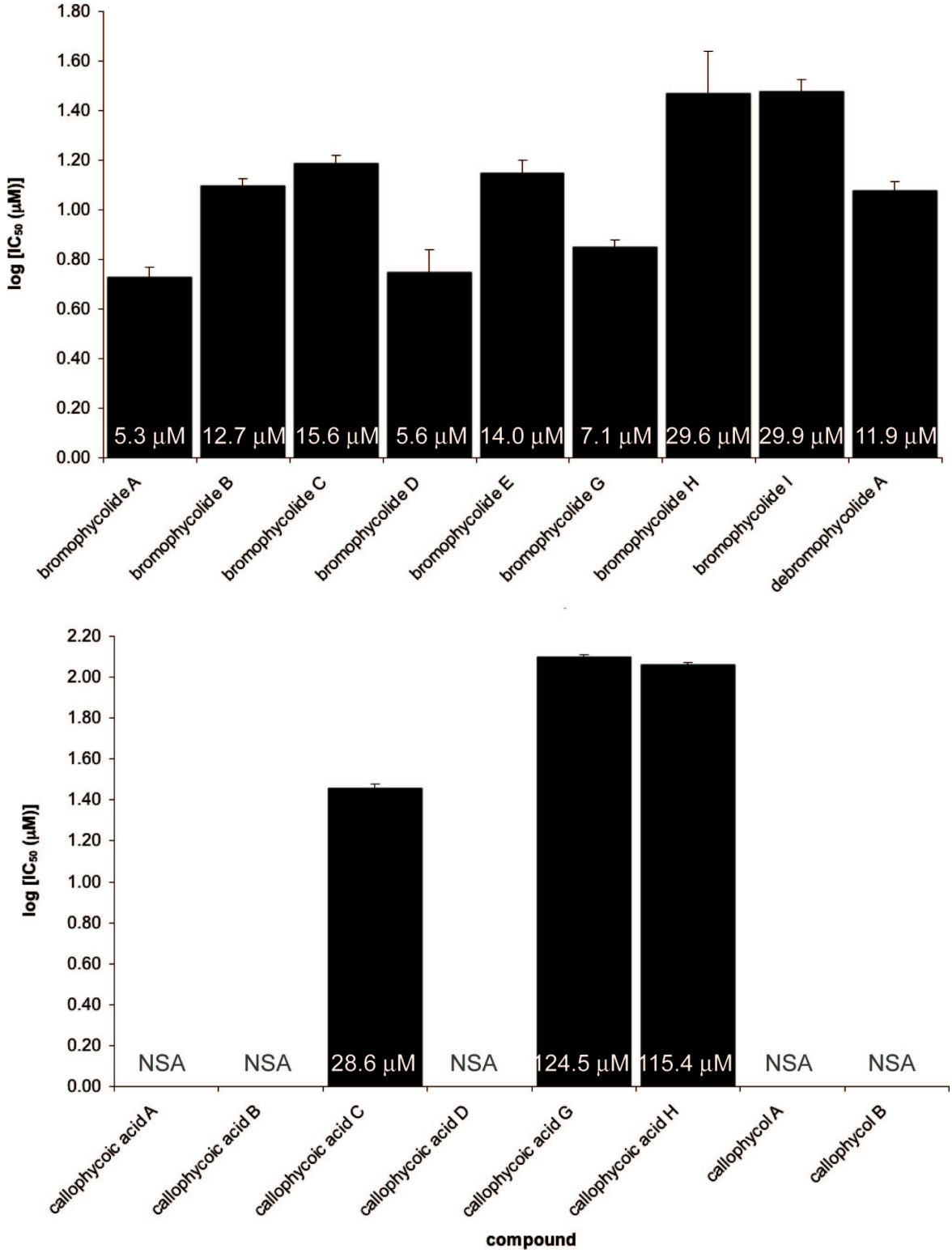

Fig. S2. Experimental log IC_{50} values for growth inhibition of the fungus *L. thalassiae*, as determined by analysis of dose-response curves. Error bars represent 1 SD. IC_{50} values are indicated in white text within each data bar. NSA denotes compounds that were not significantly active at the maximum evaluated concentration of 300 μM ($P > 0.05$, $n = 3$, 1-way ANOVA with Dunnett's post test).

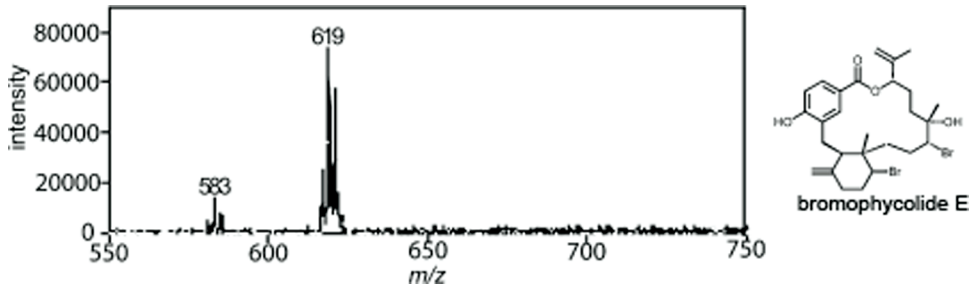

Fig. S3. Negative-ion DESI mass spectrum of pure bromophycolide E (10 μL, 1 mg/mL). The ion cluster centered at *m/z* 583 represents the $[M-H]^-$ molecular ion and *m/z* 619 represents the $[M+Cl]^-$ chloride adduct of bromophycolide E.

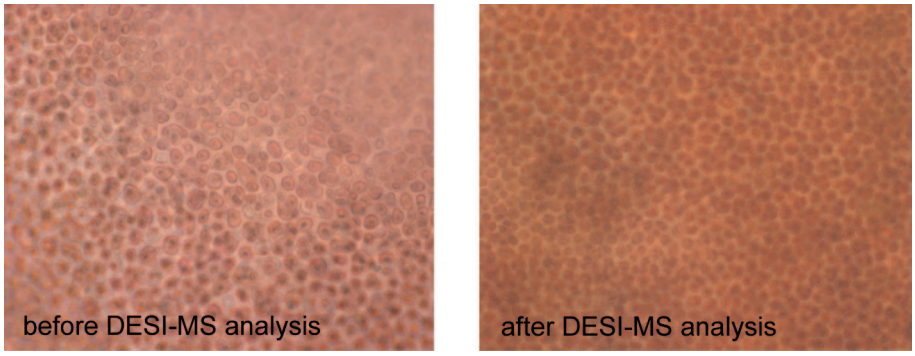

Fig. S4. Light micrographs (100× magnification) of representative *C. serratus* fragment before (*Left*) and after (*Right*) DESI-MS analysis, indicating no obvious cell lysis resulting from exposure to the DESI source.

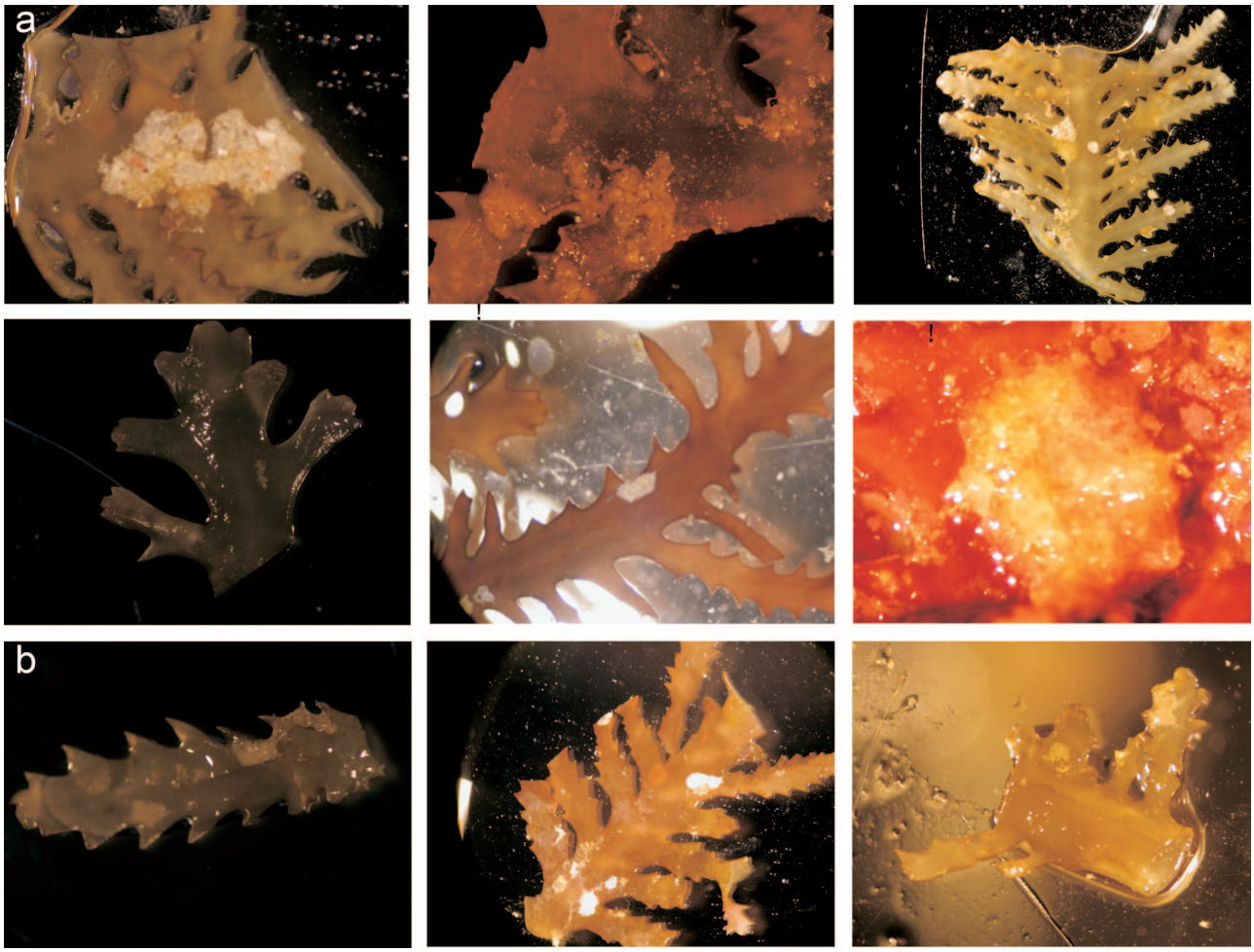

Fig. S5. Light micrographs of bromophycolide-containing patches observed on intact *C. serratus* surfaces. (*a*) Micrographs of specimens preserved with 1–10% aqueous formalin. (*b*) Images of algal samples stored at −20 °C before analysis. Patches were observed on all morphological features of the algal thallus.

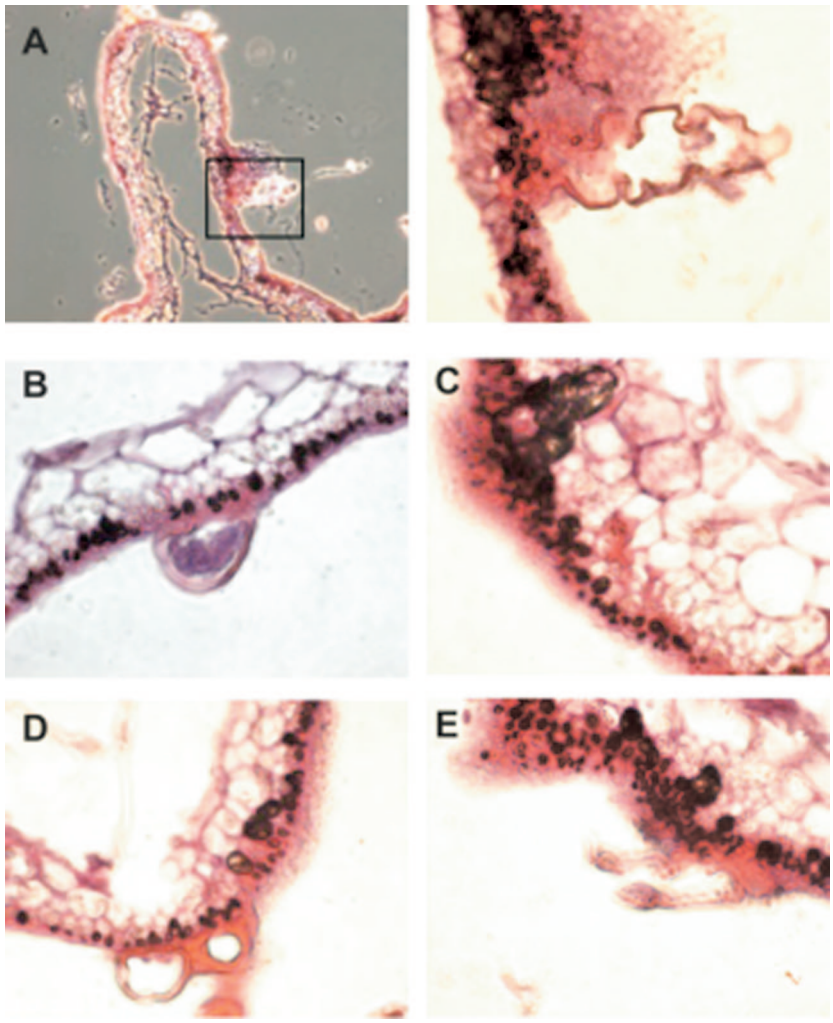

Fig. S6. Light micrographs of 5-μm sections from *C. serratus* fragments associated with bromophycolide-containing patches (preserved with 1% formalin). In all cases, sample processing dislodged patches from algal surfaces. (*A*) (*Left*) 100× magnification of *C. serratus* section. (*Right*) 400× magnification of region highlighted by black box in *A*. (*B–E*) Typical 400× micrographs of *C. serratus*, illustrating that patched regions were not associated with large-scale tissue damage. External algal surfaces are pointed downward.

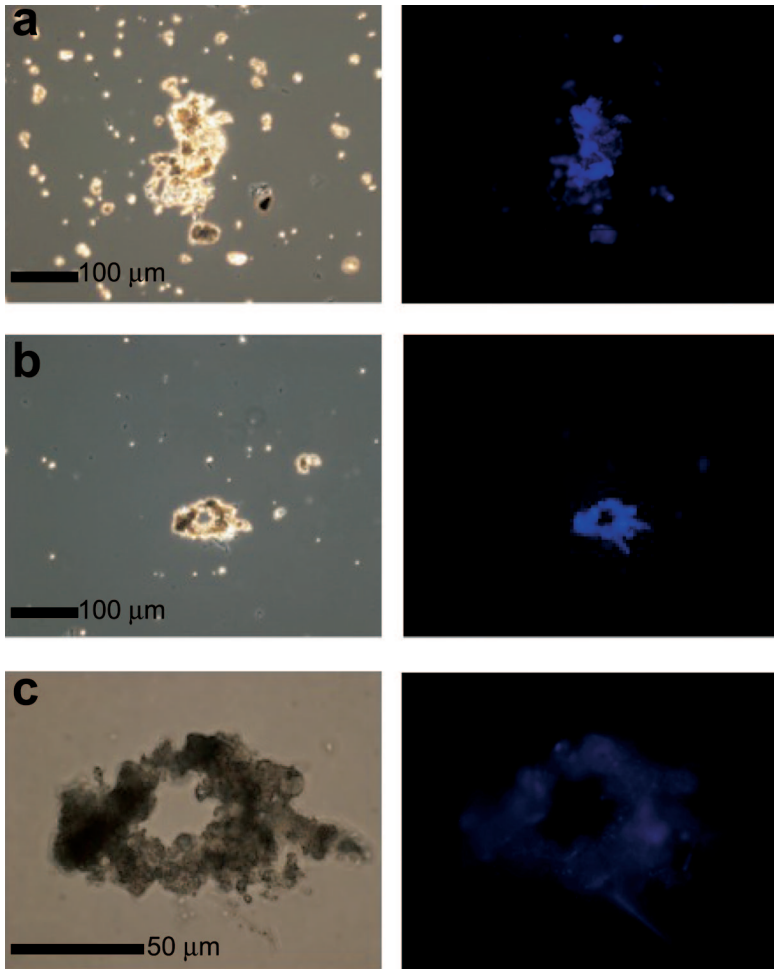

Fig. S7. Light and epifluorescence micrographs of bromophycolide-containing patches from *C. serratus* surfaces (preserved with 1% formalin and pulverized before analysis). (*a* and *b*) 100× magnification light (*Left*) and epifluorescence (*Right*) micrographs of DAPI-stained patches removed from algal surfaces. (*c*) 400× magnification light (*Left*) and epifluorescence (*Right*) micrographs of DAPI-stained patches corresponding to 100× image from *b*.

Table S1. *Callophycus serratus* collection sites in Fiji and whole tissue natural concentrations of known secondary metabolites

		Whole tissue concentration, μM																			
		Bromophycolide										Callophycoic acid								Callophycol	
ID	Collection site coordinates	A	B	C	D	E	F	G	H	I	deA*	A	B	C	D	E	F	G	H	A	B
G004	18°23′57″S177°57′58″E	151	102	46.0	22.3	22.8	ND	40.8	31.2	50.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G021	18°22′47″S177°59′37″E	165	107	24.0	10.0	35.0	ND	42.0	25.0	34.0	22.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G039	18°22′43″S177°59′41″E	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.9	10.2	257	181	ND	ND	187	48.1	61.9	23.8
G049	18°22′88″S177°58′94″E	117	81.2	20.9	120	27.9	ND	30.4	29.6	19.5	8.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G052	18°22′35″S177°59′72″E	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.1	252	131	ND	ND	241	78.4	92.5	33.3
G091	16°48′97″S179°50′84″E	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	149	188	ND	ND	27.1	20.2	75.5	25.8
G100	16°52′31″S179°52′68″E	103	146	37.9	17.7	26.1	ND	18.4	17.8	12.5	47.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
G113	18°42′49″S178°32′35″E	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	174	160	ND	ND	8.3	ND	85.1	33.0
G118	18°41′62″S178°30′72″E	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	261	178	ND	ND	113	93	72.4	25.9
G171	18°23′57″S177°57′58″E	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	129	240	ND	ND	ND	ND	178	53.1

Concentrations were determined by quantitative LC-MS of extracts from fresh plant material, and illustrate that bromophycolides and callophycoic acids/callophycols do not cooccur in individual specimens. ND denotes compounds that were not detected by selected ion recording ESI-MS. *deA denotes debromophycolide A.