

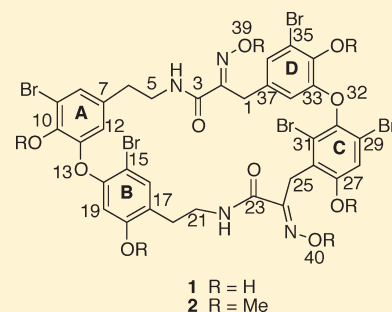
Lithothamnin A, the First Bastadin-like Metabolite from the Red Alga *Lithothamnion fragilissimum*

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S Supporting Information

ABSTRACT: Lithothamnin A (**1**) is a new bastadin-like metabolite and represents the first report of this class of molecules from the red alga *Lithothamnion fragilissimum*. Lithothamnin A contains several novel structural features that distinguish it from other bastadins. These unique structural features include novel aromatic substitution patterns and the presence of a *meta–meta* linkage between aromatic rings, in addition to the *meta–para* linkage seen in the bastadins. Lithothamnin A is modestly cytotoxic in a panel of six human tumor cell lines.



The bastadins are compounds derived from four tyrosine units that are usually highly brominated. The biosynthesis of these compounds presumably comprises the dimerization of two tyrosines to give hemibastadins, followed by either formation between two hemibastadins to produce the linear bastadins or a second ether formation to generate the macrocyclic bastadins, which are the most common. The cyclization process occurs through one of two possible oxidative phenolic couplings: as a reaction of the *para* hydroxy group of ring A with a brominated carbon on ring B to produce bastadins or a reaction involving a brominated position on ring A and the *para* hydroxy group of ring B to produce isobastadins (Figure 1).^{1,2}

All of the bastadins to date have been isolated from sponges, primarily from the genus *Ianthella*,^{3–7} with two analogues from *Psammaplysilla*.^{8,9} Numerous biological activities have been reported for the bastadins. Recent examples of these activities include cytotoxicity,³ selective binding to δ -opioid receptors,⁴ inhibition of endothelial cell proliferation,^{10,11} angiogenesis,¹² and inhibition of Ca^{2+} release from the sarcoplasmic reticulum.¹³

From the organic extract of *Lithothamnion fragilissimum* collected in Palau we isolated lithothamnin A (**1**), the first bastadin-like analogue isolated from a red alga. The few chemical studies of *Lithothamnion* reported have described oxylipins¹⁴ and phytohormones.¹⁵

Purification of the compound from the organic extract of *L. fragilissimum* using solvent–solvent partitioning,¹⁶ followed by a gel permeation column with Sephadex LH-20 and then reversed-phase HPLC, afforded a light yellow compound (**1**). The ESI mass spectrum of **1** showed an ion cluster at m/z 1047, 1049, 1051, 1053, 1055, and 1057 with intensities 9:45:100:100:47:9, consistent with the presence of five bromine atoms. The molecular formula was determined to be $\text{C}_{34}\text{H}_{27}\text{Br}_5\text{N}_4\text{O}_{10}$ by HRFABMS (CsI-doped), measuring the exact mass of a central

peak of the isotopic cluster of the Cs^+ adducts corresponding to $\text{C}_{34}\text{H}_{27}^{79}\text{Br}_3^{81}\text{Br}_2\text{CsN}_4\text{O}_{10}$, indicating the presence of 21 unsaturations.

The ¹H NMR spectrum of **1** in pyridine-*d*₅ (Table 1) exhibited two broad signals that belonged to exchangeable protons (δ_{H} 8.55 and 9.21) and signals for seven aromatic protons, three of which appeared as singlets (δ_{H} 7.23, 7.48, and 6.85) and four as doublets with *meta* coupling (δ_{H} 7.81, 7.21, 7.17, and 6.78), accounting for nine protons. The upfield region of the spectrum (δ_{H} 5.0–2.5) also contained 11 signals integrating for 12 protons (four doublets, one triplet, and six multiplets). Together these signals accounted for 21 of the 27 protons present in the molecular formula, suggesting the presence of six additional exchangeable protons. The ¹³C NMR spectrum (Table 1) contained signals for all 34 carbons. The resonances included two carbonyls, 26 additional sp^2 carbons in the range δ_{C} 160–100, and six signals for aliphatic carbons.

Analysis of 2D NMR experiments, including COSY, HSQC, HMBC, and ROESY, allowed the elucidation of four structural fragments, each containing a highly substituted aromatic ring (labeled A–D). COSY correlations between the signals at δ_{H} 7.21 (H-8, δ_{C} 128.8) and 6.78 (H-12, δ_{C} 119.0) assigned both protons to the same aromatic ring (ring A), and both protons were correlated to a carbon at δ_{C} 34.9 (C-6), suggesting that the protonated carbons were adjacent to an alkyl chain. Carbon chemical shifts and additional HMBC correlations from δ_{H} 7.21 (H-8) to C-9 (δ_{C} 112.4) and C-12 (δ_{C} 119.0) and from δ_{H} 6.78 (H-12) to C-8 (δ_{C} 128.8) and C-10 (δ_{C} 146.5) suggested that this ring had four substituents: two oxygens (δ_{C} 146.5, C-10; 147.0, C-11), a bromine (δ_{C} 112.4, C-9), and an alkyl side chain.

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While no HMBC correlations were observed for C-11 in compound **1** (δ_C 147.0), strong correlations from H-12 (δ_H 6.64) to C-11 (δ_C 151.9) were observed in HMBC experiments optimized for 3 and 8 Hz in compound **2** (see Supporting Information and below). The alkyl chain was determined to be an ethyl fragment by COSY correlations observed between the

methylene group (δ_H 3.65 and δ_H 3.32, H-5) and a second methylene group (δ_H 2.71, H-6). The H-5 signals were further correlated to the exchangeable proton at δ_H 8.55 (H-4). HMBC data further extended the ethyl group with an amide functionality based on correlations to the amide carbonyl at δ_C 165.0 from the exchangeable NH proton (δ_H 8.55, H-4).

In similar fashion, ring B was identified as an aromatic ring with two oxygen substituents, a bromine, and an ethylamide side chain, but in this fragment, HMBC correlations from the singlet aromatic proton at δ_H 7.48 (H-16) to C-14 (δ_C 158.2), C-15 (δ_C 102.6), C-17 (δ_C 125.2), C-18 (δ_C 153.2), and C-20 (δ_C 30.8) and from a singlet aromatic proton at δ_H 6.85 (H-19) to C-14 (δ_C 158.2), C-15 (δ_C 102.6), C-17 (δ_C 125.2), and C-18 (δ_C 153.2) suggested that the protons were *para* to each other, which was consistent with the lack of observed coupling between the protons.

The structure of ring C was identified as a pentasubstituted aromatic ring with an isolated methylene, two bromines, two oxygen substituents, and a single proton at δ_H 7.23. The side chain was determined to be a methylene-oxime group [δ_H 4.44 (1H, d, $J = 13.7$ Hz), 4.87 (1H, d, $J = 13.7$ Hz); δ_C 28.3 (CH₂), 152.8 (C)].

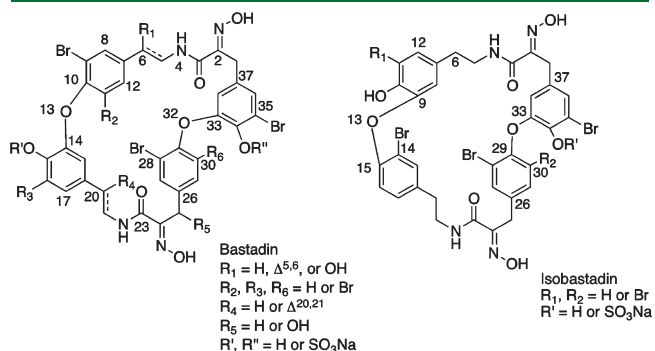


Figure 1. Summary structures of cyclic bastadins. Modified from ref 3.

Table 1. NMR Spectroscopic Data (600 MHz for ¹H and 150 MHz for ¹³C, d₅-pyridine) for Lithothamnin A (**1**)

atom no.	δ_C	δ_H mult (J in Hz)	¹ H– ¹ H COSY	HMBC 8 Hz ^a	HMBC 3 Hz ^a
1	29.5, CH ₂	a 4.10, d (12.1) b 4.19, d (12.1)	1b 1a	3, 37 37	2, 3, 36, 37, 38 2, 3, 36, 37, 38
2	152.4, C				
3	165.0, C				
4		8.55, br s	5a, 5b	3, 5	3, 5 (w) ^b
5	41.6, CH ₂	a 3.32, m b 3.65, m	5b, 6, NH-4 5a, 6, NH-4		7
6	34.9, CH ₂	2.71, t (6.7)	5a, 5b	5, 7, 8, 12	5 (w), 7, 8, 12
7	132.4, C				
8	128.8, CH	7.21, d (1.9)	6, 12	6, 7, 9, 12	5 (w), 6, 9, 12
9	112.4, C				
10	146.5, C				
11	147.0, C				
12	119.0, CH	6.78, d (1.9)	8	6, 8, 9 (w), 10	
14	158.2, C				
15	102.6, C				
16	132.4, CH	7.48, s	20a, 20b	14, 15, 18, 19 (w), 20, 21 (w)	14, 15, 17, 18, 19, 20, 21
17	125.2, C				
18	153.2, C				
19	108.3, CH	6.85, s		14, 15, 17, 18, 20 (w)	11, 14, 15, 17, 18, 20
20	30.8, CH ₂	a 3.04, m b 3.24, m	16, 20b, 21a, 21b 16, 20a, 21a, 21b		17
21	40.8, CH ₂	a 3.83, m b 3.89, m	20a, 20b, 21b 20a, 20b, 21a		
22		9.21, br s			
23	165.5, C				
24	152.8, C				
25	28.3, CH ₂	a 4.44, d (13.7) b 4.87, d (13.7)	25b 25a	24, 26, 27, 31 24, 26, 31	23, 24, 27 23, 24, 26, 27
26	128.3, C				
27	156.0, C				
28	119.6, CH	7.23, s		26, 29, 30	25
29	115.5, C				
30	141.8, C				
31	123.5, C				
33	147.0, C				
34	144.4, C				
35	111.8, C				
36	127.5, CH	7.81, d (1.7)		1, 33 (w), 34, 35, 37 (w), 38	1 (w), 33, 34, 35, 37
37	130.0, C				
38	115.5, CH	7.17, d (1.7)		1, 33, 34, 35 (w), 36	1 (w), 33, 34, 35, 36

^a HMBC correlations, optimized for 8 or 3 Hz (as indicated), are from proton(s) stated to the indicated carbon. ^b (w) indicates a weak correlation.

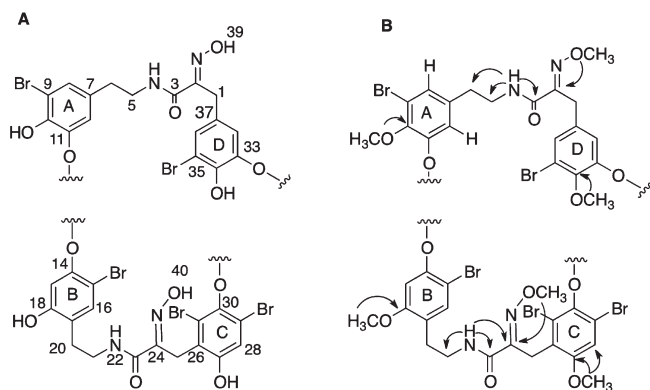
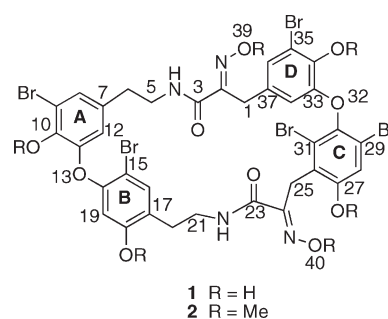


Figure 2. (A) Northern (rings A and D) and southern (rings B and C) hemispheres of lithothamnins A (1). (B) Selected HMBC correlations for the hexamethyl derivative (2) and by analogy the hydroxy groups in 1 as well as the two amide bonds present. For a listing of all observed correlations, see Table 1 for 1 and the Supporting Information for 2. For a graphic depiction of all HMBC correlations observed optimized for 8 or 3 Hz see the Supporting Information.

The last ring (D) also contained a methylene-oxime side chain, but in this case, the aromatic ring had the same substitution pattern as ring A based on carbon chemical shift and HMBC data. The two aromatic protons (δ_{H} 7.81, H-36; 7.17, H-38) both appeared as doublets with a small coupling constant (1.7 Hz), suggesting a *meta* relationship on the ring. Like ring A both protons were correlated in HMBC experiments to C-1 (δ_{C} 29.5), placing them adjacent to the side chain. Additional HMBC correlations were observed from δ_{H} 7.81 (H-36) to C-34 (δ_{C} 144.4), C-35 (δ_{C} 111.8), C-37 (δ_{C} 130.0), and C-38 (δ_{C} 115.5) and from δ_{H} 7.17 (H-38) to C-33 (δ_{C} 147.0), C-34 (δ_{C} 144.4), and C-36 (δ_{C} 127.5). These four rings accounted for everything required by the molecular formula except four hydrogen atoms and one of 21 unsaturations. Assuming that these hydrogen atoms would be placed on oxygens, it suggested that compound 1 was a macrocyclic derivative, related to the bastadin family of compounds (Figure 1).

To determine the position of the hydroxy groups on each ring and therefore the ether linkages, compound 1 (2.2 mg) was methylated with methyl iodide. The KCl-doped FABMS spectrum of the reaction product showed an ion cluster at m/z 1169, 1171, 1173, 1175, 1177, and 1179, suggesting the addition of six CH_2 plus potassium. The molecular formula was determined to be $\text{C}_{40}\text{H}_{39}\text{Br}_5\text{N}_4\text{O}_{10}$ by HRFABMS (CsI-doped) of one of the central peaks of the isotopic cluster. The ^1H NMR spectrum of 2 (Supporting Information) contained six singlets between δ_{H} 3.7 and 4.2, which integrated for three protons each, indicating the presence of a hexamethyl derivative and confirming the presence of six hydroxy groups in 1 (including the two oximes). Four oxygenated methyl signals in 2 showed three-bond correlations in a HMBC experiment optimized for 8 Hz (δ_{H} 4.08 to δ_{C} 146.2, C-10; δ_{H} 3.58 to δ_{C} 159.1, C-18; δ_{H} 3.71 to δ_{C} 157.0, C-27; and δ_{H} 4.13 to δ_{C} 145.2, C-34), placing four of the methyl groups and therefore the corresponding hydroxy groups at these positions (C-10, C-18, C-27, and C-34) in 1. A second HMBC optimized for 3 Hz showed four-bond correlations from the remaining methoxy protons at δ_{H} 3.93 to the oxime carbon at δ_{C} 152.1 (C-2) and from δ_{H} 3.73 to δ_{C} 152.8 (C-24). This experiment also showed four-bond correlations from δ_{H} 3.58 (OCH_3 -18) to δ_{C} 106.1 (C-19) and from δ_{H} 3.71 (OCH_3 -27) to δ_{C} 115.5 (C-28), providing additional support for the placement of the methoxy groups in 2 and therefore the hydroxy groups at these positions in 1 (Figure 2 and Supporting Information).

The amide linkage between rings A and D was revealed by correlations from δ_{H} 4.10 (H-1a) to δ_{C} 165.0 (C-3) in compound 1 and from 8.57 (NH-4) to δ_{C} 163.6 (C-3) and 41.6 (C-5) in 2 (Figure 2B). In a similar fashion, HMBC correlations from δ_{H} 4.44 (H-25a) and 4.87 (H-25b) to δ_{C} 165.5 (C-23) and δ_{C} 152.8 (C-24) in 1 were extended in the hexamethyl derivative 2 (Figure 2B, and Supporting Information) with correlations from NH-22 (δ_{H} 8.97) to δ_{C} 163.8 (C-23) and δ_{C} 40.3 (C-21), which connected rings B with C through a second amide-oxime system (Figure 2B) to generate a northern and southern hemisphere for 1. The northern hemisphere looks very “bastadin-like” as a condensation between brominated tyrosine and brominated tyramine units. In contrast, the southern hemisphere of lithothamnins A consists of a condensation of a 9-bromo 5,8-dihydroxyphenylalanine and a 5,7-dibromo-4,6-dihydroxyphenethylamine. Hydroxylation at the 2-position has been reported as part of the synthesis of spiroxazolines,^{17,18} but has not been observed previously in bastadins.



Because both the A and D rings had two adjacent oxygen substituents, unequivocal assignment of the hydroxy versus ether links was challenging given the low number of protons on the aromatic rings. One can envision two possibilities for each. For example, rings A and B could be linked from the oxygen on C-11 in A to the C-14 in B, resulting in a “*meta-para*” linkage based on the position of the ether link relative to the respective side chains. Alternatively if the link were from C10 to C-14, it would result in a “*para-para*” link. Similarly one could link rings C and D from C-30 to C-34 (*meta-para*, Figure 3B) or from C-30 to C-33 (*meta-meta*, Figure 3A). To date, all of the cyclic bastadins contain only *meta-para* links.³

Using NMR prediction software (ChemDrawPro 12.0 Mac) we compared the predicted carbon values for each structure with the experimental values we obtained for both lithothamnins A (1) and the hexamethyl derivative 2. The values for the C and D rings are found in the tables of Figure 3 (see Supporting Information for A and B ring data). The predicted NMR values for linking rings C and D through C-30 and C-33 (*meta-meta*, Figure 3A) consistently matched the measured values more closely than those for a link between C-30 and C-34 (*meta-para*, Figure 3B). Similarly, the NMR resonances observed with both the natural product 1 and the methyl derivative 2 matched the predicted chemical shifts of the “*meta-para*” linkage between fragments A and B, corresponding to an ether link between C-11 and C-14 (Supporting Information). Additional support for the ether link between C-11 and C-14 was the observed HMBC correlation from H-19 to C-11 observed for 1 in the experiment optimized for 3 Hz.

The configurations of both oxime groups were assigned as *E* by analysis of their ^{13}C NMR data. Chemical shifts of δ_{C} 29.5 (C-1) and δ_{C} 28.3 (C-25) are characteristic for *E* oximes, in contrast to the lower field value (~ 36 ppm) expected for *Z* isomers.³

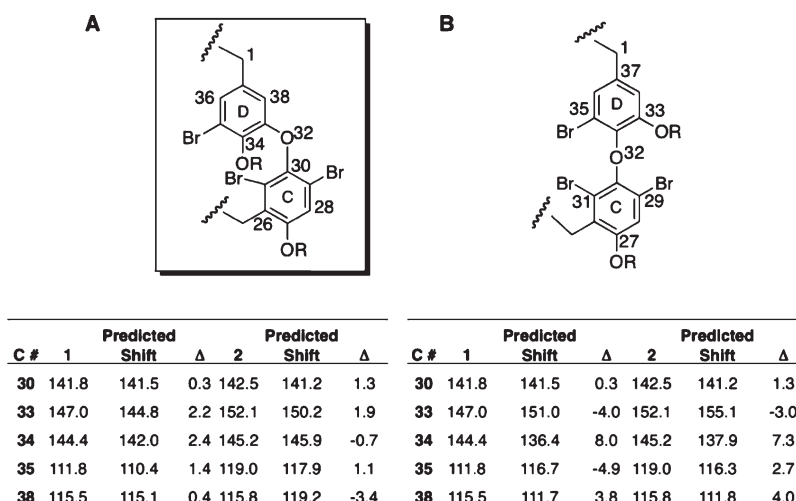


Figure 3. Comparison of the possible ether linkages between rings C and D [panels A (*meta*–*meta*) and B (*meta*–*para*)] with the predicted and observed ^{13}C NMR values and the calculated difference (Δ) for the ring carbons for both lithothamnin A (**1**, R = H) and the hexamethyl derivative (**2**, R = CH_3). The boxed structure (A) indicates the structure that best matches observed and predicted chemical shifts. A similar analysis for the linkage between rings A and B is available in the Supporting Information.

Comparing the structure of lithothamnin A to the bastadin family there are several noticeable differences. While the northern hemisphere (rings A and D) in **1** is identical to that of bastadins **19**^{2,19} and **20**,² the southern hemisphere of lithothamnin A is unprecedented among the bastadins. In addition to the unique *meta*–*meta* linkage between rings C and D, another feature that distinguishes this compound from other cyclic bastadins is the presence of 2-hydroxy substituents on the B and C rings. However, ring C is present in clavatine A, isolated from the sponge *Suberea clavata*.²⁰ The reported NMR values of clavatine A and ring C of **1** are very similar (see Supporting Information). However, no structure analogous to ring B has been reported.

A third observed difference is the isolated methylenes (C-1 and C-25) in the molecule that appear in the ^1H NMR spectrum of **1** as diastereotopic pairs (δ_{H} 3.32, 3.65 H-1; δ_{H} 4.44, 4.87 H-25) instead of appearing as a two-proton singlet as seen in other cyclic bastadins. The corresponding diastereotopic methylene signals have been observed only in bastadins **8** and **9**.²¹ In the case of lithothamnin A, the diastereotopic signals for H-1 and H-25 are present in a variety of solvents and at temperatures as high as 45 °C, and they show characteristic vicinal couplings in the COSY spectrum (see Supporting Information), indicating this is not due to the presence of atropisomers. In addition, the optical rotation for **1** was essentially zero, and the CD spectrum was a flat line.

A molecular model of lithothamnin A was constructed, and energy minimization, using MM2 and semiempirical methods (AM1/PM3), was performed using CS ChemBio3D Ultra. On the basis of these studies, the *meta*–*meta* coupling between the C and D rings and the presence of two bromines that flank the ether link on ring C impart enough rigidity to that portion of the molecule such that the phenyl rings do not rotate. In addition, each proton of the methylenes at C-1 and C-25 lies on opposite sides of the macrocycle, resulting in the observed difference in the chemical shifts for each proton.

Lithothamnion is an encrusting red algal species from the family Hapalidiaceae in the order Corallinales that has rarely been chemically studied.^{14,15} While bromophenols are widely reported from red algae,²² they do not generally reach the structural complexity of the bastadins. There is no evidence that

our source material is a mixed collection of sponge and algae. No sponge material was noted in the collection notes (as required by the collection contract; see Supporting Information) or during multiple taxonomic evaluations of the voucher. Finally, lithothamnin A is found in the same yield or concentration as many of the bastadins isolated to date (see Supporting Information), which would suggest that if the producing organism was a sponge contaminant, it would have to produce this new and unusual bastadin in very high yield and the sponge material would have to be a major contributor to the extracted material. Isolation of a bastadin-like compound from a Rhodophyte may suggest that the ultimate producer of these compounds is microbial and may also suggest that these rarely chemically studied organisms harbor more chemical diversity than generally thought.

Lithothamnin A (**1**) was tested against six different human tumor cell lines but exhibited only modest antiproliferative activities against five of the six cell lines [cell line (IC_{50} in μM): LOX (9.5), SNB-19 (7.6), OVCAR-3 (7.6), COLO-205 (19.0), MOLT-4 (19.0)].

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Beckman DU 640 spectrophotometer, the CD spectrum was obtained on a Jasco J-720 spectropolarimeter, and IR spectra were obtained on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. NMR spectra were obtained on Varian Inova Unity 500 and Bruker Avance III 600 spectrometers in $\text{DMSO}-d_6$ and pyridine- d_5 . High-resolution mass spectra were acquired on a JEOL SX102 mass spectrometer, and CsI was added to the samples prior to analysis. Electrospray ionization mass spectra were recorded on a Hewlett-Packard HP1100 integrated LC-MS system equipped with an ion spray interface. HPLC separations utilized a Varian ProStar system with a Rainin Dynamax UV-C detector.

Algal Material. *Lithothamnion fragilissimum* (Corallinaceae, Rhodophyceae) was collected 4 km east of Lighthouse Reef, Palau Island, in September 1993, by P. Colin (Coral Reef Research Foundation) under contract with the National Cancer Institute. A voucher specimen (0CDN1665) has been deposited at the Smithsonian Institution. The taxonomy was determined by G. Trono (U. Philippines).

Extraction and Isolation. The frozen material were stored at $-20\text{ }^{\circ}\text{C}$ until extracted at the National Cancer Institute as described by McCloud.²³ Briefly, frozen algal material was ground in the presence of dry ice and then extracted with water to form an aqueous extract. Following lyophilization, the algal material was then extracted with CH_2Cl_2 –MeOH (1:1) overnight and then extracted a second time with MeOH for 30 min. The resulting organic extracts were combined, and solvent was removed using rotary evaporation. The remaining solvent was removed under high vacuum to generate the organic extract. A portion (1.13 g) of the organic extract of *L. fragilissimum* was subjected to solvent–solvent partitioning.¹⁶ The MeOtBu fraction was subjected to gel permeation chromatography on Sephadex LH-20 (CH_2Cl_2 –MeOH, 1:1) to give 11 fractions (A–K). The last fraction (K) contained compound **1**. Final purification of **1** was achieved by reversed-phase C_{18} HPLC (1:4 MeOH– H_2O to 100% MeOH over 25 min; hold 5 min) to yield 3 mg (0.28% of extract).

Lithothamnin A (1): light yellow solid; UV (MeOH) λ_{max} (log ϵ) 226.0 (4.5), 287.5 (3.9) nm; IR (film) ν_{max} 3272, 2924, 2848, 1661, 1581, 1535, 1496, 1423, 1277, 1210, 1175, 1022 cm^{-1} ; ^1H and ^{13}C NMR in pyridine- d_5 , see Table 1; ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) in DMSO- d_6 , see S-8 in the Supporting Information; HRFABMS (magic-bullet, doped with CsI) m/z 1182.6671 (calcd for $\text{C}_{34}\text{H}_{27}^{79}\text{Br}_3^{81}\text{Br}_2\text{CsN}_4\text{O}_{10}$ 1182.6659); FABMS (magic-bullet) m/z 1069–1079 $[\text{M} + \text{Na}]^+$, 1047–1057 $[\text{M} + \text{H}]^+$.

Hexa-O-methylthiothamnin A (2). To a solution of compound **1** (2.2 mg) in DMF (1 mL) were added 100 mg of K_2CO_3 and methyl iodide (380 μL). The reaction was stirred at rt and protected from light. After 43 h, CH_2Cl_2 was added, the reaction mixture was filtered off, and the solvent was removed under reduced pressure, affording a yellow oil: ^1H NMR and ^{13}C NMR (pyridine- d_5 and DMSO- d_6 , 600 MHz) see S-9 and S-10, respectively, in the Supporting Information; HRFABMS (magic-bullet, doped with CsI) m/z 1268.7554 (calcd for $\text{C}_{40}\text{H}_{39}^{79}\text{Br}_2^{81}\text{Br}_3\text{CsN}_4\text{O}_{10}$ 1268.7572); FABMS (magic-bullet, doped with KCl) m/z 1169–1179 $[\text{M} + \text{K}]^+$.

In Vitro Cytotoxicity Assay. The in vitro six-cell-line bioassay was a 2-day bioassay. Cells were grown in RPMI-1640 without L-glutamine, supplemented with 10% fetal bovine serum, 5.0 mL of a 200 mM glutamine stock, and 0.5 mL of gentamicin, and plated out in T-162 cm^2 flasks. Once the cells were confluent, they were harvested and plated in 96-well microtiter flat-bottom plates at a seeding density of 50–100 000 cells per well, to yield optical density readings in the range 1–2.0, and incubated for 1 h in a 37 $^{\circ}\text{C}$, 5% CO_2 incubator. After the 1 h incubation, the cells were then introduced to the test sample via a Beckman Biomek Workstation-1000. The Biomek-1000 performed eight serial dilutions in a 96-well round-bottom plate and then transferred aliquots of 100 μL to the assay plate. The plate was then returned to the incubator for 24 h. After the 2-day incubation, the cells were exposed to a tetrazolium salt (2,3-bis[2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide) for a 4 h incubation in a 37 $^{\circ}\text{C}$ incubator, where metabolically viable cells reduced the tetrazolium salt to a colored formazan product. Once the incubation was completed, the plates were then read in a dual-wavelength mode at 450 nm, with a 650 nm reference, using a SpectraMAX 250 (Molecular Devices) plate reader. A positive control of 20% DMSO was used.

■ ASSOCIATED CONTENT

Supporting Information. Pictures of the organism. NMR spectra for lithothamnin A in pyridine- d_5 (^1H , ^{13}C , COSY, HSQC, HMBC) and ^1H NMR spectrum of hexamethylthiothamnin A in pyridine- d_5 and tables of ^1H , ^{13}C NMR data for **1** and **2** in DMSO- d_6 and for **2** in pyridine- d_5 . Structure drawing detailing HMBC correlations seen optimized for 8 or 3 Hz. Predicted and observed NMR values for ether link between rings A and B. ^1H NMR spectra of the variable-temperature

experiments of **1** and an expansion of the region incorporating H-1 and H-25 of the COSY obtained at 45 $^{\circ}\text{C}$. Comparative yields of bastadins. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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