

Antifungal Alkyl Amino Alcohols from the Tropical Marine Sponge *Haliclona n. sp.*

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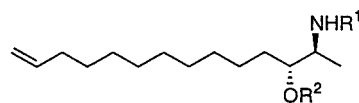
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Three new amino alcohols presumably deriving from L-alanine were isolated from the tropical marine sponge *Haliclona n. sp.* and characterized by 2D NMR, while a fourth amino alcohol was characterized as an acetamide derivative. Relative stereochemistry was deduced from the NMR characteristics of oxazolidinone derivatives and absolute stereochemistry secured by preparation and analysis of an MPA ester. The amino alcohol fraction from *Haliclona n. sp.* acts as an antifungal agent and inhibits the development of larvae of the ascidian *Herdmania curvata*.

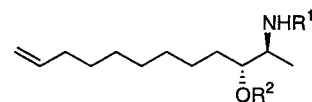
The typical metabolites of sponges of the genus *Haliclona* are alkaloids,^{1,2} including amino alcohols derived from serine,³ alanine,⁴ and glycine.⁵ A striking orange to deep orange-red sponge, identified as *Haliclona n. sp.*, was collected at Heron Island on the Great Barrier Reef at a depth of approximately 10–15 m. The CH₂Cl₂/MeOH (1:1) extract of the sponge was partitioned between EtOAc and H₂O, then purified with EtOAc/MeOH on a normal phase flash column to give a major amino alcohol fraction. This fraction, shown to consist of several compounds by NMR analysis, was further purified by RPHPLC (C₁₈; MeOH/H₂O with 0.2% TFA) to give the three amino alcohols, halaminols A–C. Subsequently a fourth amino alcohol, halaminol D, was isolated as its acetamide derivative.

The molecular formula of the major component **1a**, named halaminol A, was determined as C₁₄H₂₉NO by HREIMS. The ¹H NMR spectrum (Table 1) contained characteristic vinyl ABX resonances (three one-proton signals at δ 4.90 (dd), 5.01 (dd) and 5.79 (tdd)). Signals at δ 3.69 (m) and 3.26 (m) were consistent with protons next to oxygen and nitrogen respectively, and there was also a methylene envelope (δ 1.4–1.5) as well as a methyl doublet at δ 1.20. The ¹³C spectrum supported this information with signals for two alkene carbons (δ 140.1 and 114.7 ppm), methine carbons next to oxygen and nitrogen (δ 71.7 and 52.6), nine methylenes, and a methyl group (δ 12.0). The structure of **1a** was assigned as a linear amino alcohol with the use of DQFCOSY, TOCSY, geHSQC, and geHMBC data. Halaminol B, **2a**, the second most abundant metabolite, had a molecular formula of C₁₂H₂₅NO by HREIMS, a proton spectrum almost identical to that of **1a**, and a ¹³C spectrum with two less carbons. The structure was confirmed by analysis of DQFCOSY and HMBC data and by comparison of the data with that of **1a**. The third metabolite, halaminol C, **3a**, had a molecular formula of C₁₄H₂₉NO by HREIMS and thus was a double bond isomer of **1a**. There were two discrete one-proton resonances at δ 5.39 (td) and 5.54 (td), the characteristic downfield signals for the amino alcohol portion, a methyl doublet at δ 1.23, and a methyl triplet at δ 0.89. The ¹³C spectrum showed two alkene CH's at δ 125.2 and 134.1, two methine carbons next to oxygen and nitrogen (δ 71.7 and 51.9), plus eight methylenes, and two methyl signals (δ 11.8 and 14.4). In the COSY spectrum, a methylene signal at δ 2.49 was next

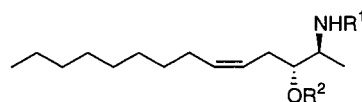
to both the alkene proton signal at δ 5.39 and the carbinol proton at δ 3.74; therefore halaminol C possessed a Δ⁵ double bond. The *Z* stereochemistry was deduced as follows: (i) the alkene hydrogens showed a *J* of 11 Hz; (ii) C-7 had a ¹³C chemical shift of δ 28.5;^{2a} (iii) the NOESY spectrum showed a correlation between H-5 and H-6.



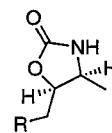
- (1a) R¹ = R² = H
 (1c) R¹ = R² = Ac
 (1d) R¹ = Ac; R² = H
 (1e) R¹ = Ac; R² = (*R*)-MPA
 (1f) R¹ = Ac; R² = (*S*)-MPA



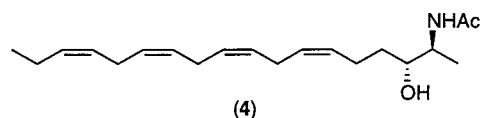
- (2a) R¹ = R² = H
 (2c) R¹ = R² = Ac
 (2d) R¹ = Ac; R² = H



- (3a) R¹ = R² = H
 (3c) R¹ = R² = Ac
 (3d) R¹ = Ac; R² = H



- (1b) R = -(CH₂)₈CH=CH₂
 (2b) R = -(CH₂)₆CH=CH₂
 (3b) R = -CH=CH-(CH₂)₆CH₃



The relative stereochemistry of the 2-amino-3-hydroxy moiety in amino alcohols can be deduced by analysis of

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Table 1. ^{13}C and ^1H NMR Data for Amino Alcohols **1a**, **2a**, and **3a**

| position | 1a | | 2a | | 3a | |
|----------|-------------------------|--|-------------------------|--|-------------------------|----------------------|
| | $\delta^{13}\text{C}^a$ | $\delta^1\text{H}^b$ | $\delta^{13}\text{C}^a$ | $\delta^1\text{H}^b$ | $\delta^{13}\text{C}^a$ | $\delta^1\text{H}^b$ |
| 1 | 12.0 (q) | 1.20 (3H, d, 7) | 12.0 (q) | 1.20 (3H, d, 7) | 11.8 (q) | 1.23 (3H, d, 7) |
| 2 | 52.6 (d) | 3.26 (1H, m) | 52.6 (d) | 3.25 (1H, m) | 51.9 (d) | 3.29 (1H, dq, 3, 7) |
| 3 | 71.7 (d) | 3.69 (1H, m) | 71.7 (d) | 3.68 (1H, m) | 71.7 (d) | 3.74 (1H, dt, 3, 6) |
| 4 | 34.0 (t) | 1.45 (2H, m) | 34.0 (t) | 1.44 (2H, m) | 32.3 (t) | 2.49 (2H, dd, 6, 7) |
| 5 | 30.64 ^c | 1.4–1.5 | 26.9 (t) | 1.52 (1H, m) 1.34 (1H, m) | 125.2 (d) | 5.39 (1H, td, 7, 11) |
| 6 | 30.64 ^c | 1.4–1.5 | 30.07 (t) ^c | 1.33 | 134.1 (d) | 5.54 (1H, td, 7, 11) |
| 7 | 30.59 ^c | 1.4–1.5 | 30.13 (t) ^c | 1.33 | 28.5 (t) | 2.08 (2H, q, 7) |
| 8 | 30.57 ^c | 1.4–1.5 | 30.47 (t) ^d | 1.36 | 30.7 (t) ^c | 1.29–1.37 |
| 9 | 30.21 ^c | 1.4–1.5 | 30.54 (t) ^d | 1.36 | 30.6 (t) ^c | 1.29–1.37 |
| 10 | 30.11 ^c | 1.4–1.5 | 34.9 (t) | 2.04 (2H, q, 7) | 30.5 (t) ^c | 1.29–1.37 |
| 11 | 30.00 ^c | 1.4–1.5 | 140.1 (d) | 5.80 (1H, tdd, 7, 11, 17) | 30.5 (t) ^c | 1.29–1.37 |
| 12 | 34.9 (t) | 2.03 (2H, q, 7) | 114.7 (t) | 4.90 (1H, dd, 2, 11) 5.01 (1H, dd, 2, 17) | 33.1 (t) | 1.27 |
| 13 | 140.1 (d) | 5.79 (1H, tdd, 7, 11, 17) | | | 23.7 (t) | 1.29 |
| 14 | 114.7 (t) | 4.90 (1H, dd, 2, 11) 5.01 (1H, dd, 2, 17) | | | 14.4 (q) | 0.89 (3H, t, 7) |

^a Detection at 125 MHz for solution in d_4 -MeOH; multiplicity by DEPT in parentheses. ^b Detection at 500 MHz for solution in d_4 -MeOH; integration, multiplicity, and J in Hz in parentheses. ^{c,d} Interchangeable.

NMR data of the oxazolidinone derivatives.^{4c} Halaminols A–C were each converted to the corresponding oxazolidinone **1b–3b** by refluxing with 1,1-carbonyldiimidazole in dry benzene and the products purified by silica chromatography. All three halaminol derivatives had a J of 7 Hz between H-2 and H-3, indicating *cis* stereochemistry, and hence that the parent amino alcohol was $2S^*$, $3R^*$. The alternative *trans* stereochemistry would have given a J of 10 Hz for the oxazolidinones.

Each halaminol metabolite had an optical rotation that was positive in sign and of small magnitude (approx $+2^\circ$). In addition the diacetate derivative (**1c–3c**) of each halaminol, prepared by Ac_2O /pyridine treatment, gave a negative value of approximately -25° . These values correspond closely with those determined by Mori and Matsuda for a synthetic sample of a $2S$, $3R$ amino alcohol and its diacetate.^{4b} Confirmation of the suggested stereochemistry was obtained by preparation and NMR analysis of an MPA ester from halaminol A. The diacetate **1c** was refluxed with K_2CO_3 in dry methanol to give the acetamide **1d**, which was stirred with either (*R*)- or (*S*)-methoxyphenylacetic acid, DCC, and DMAP, and the MPA ester product **1e** or **1f** purified by silica chromatography. When the ^1H NMR spectra of the two esters were compared, the signals for H-1 and H-2 were shifted upfield in the *R* ester relative to the *S* ester (i.e., $\delta^R - \delta^S$ was negative), while for H-4 and H-5, $\delta^R - \delta^S$ was positive. Thus the two esters are represented by Newman projections in which the absolute configuration of halaminol A is $2S$, $3R$. The ^1H NMR spectrum of the (*R*)-MPA ester of halaminol A acetamide **1e** was then measured at 300 K and at 190 K in $\text{MeOH}-d_4$. At the lower temperature, upfield shifts were observed for the H-1 and H-2 resonances (which must lie in the shielding cone of the phenyl ring), whereas the H-4 proton signals were shifted downfield. These temperature-dependent NMR shifts⁶ are in accordance with the Newman projection model.

During preliminary studies investigating the preparation of halaminol A acetamide from a mixture of halaminols, a fourth amino alcohol, halaminol D, was isolated as the *N*-acetyl derivative **4**. The molecular formula of $\text{C}_{20}\text{H}_{33}\text{NO}_2$ was measured by HREIMS, indicating four elements of unsaturation. A characteristic methylene-interrupted double bond pattern was recognized from the general appearance of the ^1H NMR spectrum. The structure of **4** was confirmed with the use of DQFCOSY, TOCSY, geHSQC,

and geHMBC data; in particular, the COSY analysis revealed the positioning of double bonds between C-6 and C-7 and between C-15 and C-16. The ^{13}C chemical shifts of the allylic methylenes were all less than δ 30, consistent with *cis* double bond stereochemistry.^{2a}

In a standard paper disk assay, halaminols A–C each inhibited the growth of the microorganisms *E. coli*, *B. subtilis*, *C. albicans*, *T. mentagrophytes*, and *C. resinae*. Halaminols A and B were the most effective, causing a 10 mm zone of inhibition on the test plate when tested against *T. mentagrophytes* and *C. resinae*. The diacetyl derivative **1c** was inactive against all microorganisms except *B. subtilis* (7 mm zone of inhibition), while acetamide **1d** was inactive against all test organisms. The biological activity exhibited by these linear amino alcohols may result from their detergent-like nature, which is likely to disrupt cell membranes. Acetylation decreases the biological activity of the halaminols, presumably by altering the surfactant properties of the metabolites. When tested at ecologically relevant concentrations against the ascidian *Herdmania curvata*,⁷ the amino alcohol fraction from *Haliclona* n. sp. induced larval settlement when compared to controls, but then disrupted the subsequent developmental processes. All larvae were judged dead 6 h after settlement. We speculate that the halaminols may therefore play a role in situ in preventing fouling of the sponge surface. Ongoing work in our research group is aimed at elucidating the molecular mechanisms involved.

Experimental Section

General Experimental Procedures. These have been reported previously.⁸

Animal Material. The sponge *Haliclona* sp. 1031 (order Haplosclerida, family Chalinidae) was collected at approximately 10–15 m depth at Heron Island ($23^\circ 27' \text{S}$, $151^\circ 55' \text{E}$) on the Great Barrier Reef. It does not conform to any of the known (described) Chalinidae from Australia or the Indo-Malay Archipelago and is likely new to science. This species appears to be restricted to the southern part of the Great Barrier Reef (Capricorn-Bunker Gp., Swain Reefs, Whitsunday Is.) and is also recorded from Fiji (QM database). The sponge is encrusting and is usually found attached to dead massive corals. In life, its coloration is a striking orange color, ranging to more deep orange-red in exposed habitats, and is one of the distinguishing features of this species. The sponge tissue is soft, spongy, and very compressible, and the sponge body begins to disintegrate when handled or collected. The surface

has prominent oscules of 3–5 mm. The spionid polychaete *Polydorella prolifera* lives in intimate association with *Haliclona* n. sp. 1031. A voucher specimen of the sponge (G312726) is held at the Queensland Museum, Brisbane.

Extraction and Isolation. The wet sponge (800 g) was extracted with 1:1 CH₂Cl₂/MeOH (4 × 400 mL). After filtration and concentration in vacuo, the residue was partitioned between EtOAc (3 × 300 mL) and H₂O (500 mL). The organic extract was then dried with MgSO₄ and evaporated to give an orange oil (17 g), which showed pronounced antibiotic and antifungal activity. The oil was passed down a silica flash column eluting with 100% EtOAc, then 30% MeOH/EtOAc, then 100% MeOH to afford the halaminols (4.9 g). Further purification of a 0.23 g portion by reversed-phase HPLC using MeOH/H₂O (80:20) containing 0.2% TFA gave three major amino alcohols, halaminols A (74 mg, 0.2% of sponge wet wt), B (27 mg, 0.08%), and C (26 mg, 0.07%), as their trifluoroacetate salts.

Halaminol A (1a): (2*S*)-aminotetradeca-13-en-(3*R*)-ol; colorless oil; [α]_D +1.7° (c 0.044, CH₂Cl₂); ¹H and ¹³C NMR, see Table 1; EIMS *m/z* 228 [MH]⁺ (60), 149 (40), 114 (100); HREIMS *m/z* 228.2329 [MH]⁺ (calcd for C₁₄H₃₀NO, 228.2322).

Halaminol B (2a): (2*S*)-aminododeca-11-en-(3*R*)-ol; colorless oil; [α]_D +2.1° (c 0.056, CH₂Cl₂); ¹H and ¹³C NMR, see Table 1; EIMS *m/z* 200 [MH]⁺ (100), 131 (40); HREIMS *m/z* 200.2009 [MH]⁺ (calcd for C₁₂H₂₆NO, 200.2009).

Halaminol C (3a): (2*S*)-aminotetradeca-(5*Z*)-en-(3*R*)-ol; colorless oil; [α]_D +1.9° (c 0.025, CH₂Cl₂); ¹H and ¹³C NMR, see Table 1; EIMS *m/z* (relative intensity) 228 ([MH]⁺, 50), 149 (40), 114 (100); HREIMS *m/z* 228.2329 [MH]⁺ (calcd for C₁₄H₃₀NO, 228.2322).

Preparation of Oxazolidinone Derivatives. Halaminol A (**1a**, 13 mg) was refluxed with 1,1-carbonyldiimidazole (45 mg) in dry benzene (2 mL) for approximately 6 h followed by stirring at room temperature overnight. The crude product was taken up in EtOAc (10 mL) and washed with H₂O (3 × 5 mL), and the organic phase was dried over MgSO₄ and then filtered. The solvent was removed by rotary evaporation and the residue (12.3 mg) put through a silica cartridge and eluted with EtOAc to give purified halaminol A oxazolidinone (**1b**, 11.0 mg, 76%). An equivalent procedure was used to prepare halaminol B and C oxazolidinone derivatives (**2b** and **3b**, respectively).

Halaminol A oxazolidinone (1b): white amorphous solid; [α]_D -23.4° (c 0.008, CH₂Cl₂); ¹H NMR (CDCl₃) δ 5.79 (1H, tdd, *J* = 6, 10 and 17 Hz, H-13), 5.30 (1H, bs, *NH*), 4.97 (1H, brd, *J* = 17 Hz, H-14a), 4.91 (1H, brd, *J* = 10 Hz, H-14b), 4.55 (1H, m, H-3), 3.88 (1H, q, *J* = 7 Hz, H-2), 2.02 (2H, q, *J* = 6 Hz, H-12), 1.72 (1H, m, H-4a), 1.51 (1H, m, H-4b), 1.50–1.20 (14H, m), 1.70 (3H, d, *J* = 7 Hz, H-1); ¹³C NMR (CDCl₃) δ 155.3 (s, C-15), 139.2 (d, C-13), 114.2 (t, C-14), 80.2 (d, C-3), 51.1 (d, C-2), 33.8 (t, C-12), 29.42 (t), 28.40 (t), 29.38 (t), 29.35 (t), 29.12 (t), 29.07 (t), 28.9 (t), 25.8 (t, C-4), and 16.0 (q, C-1); ESMS *m/z* [M + H]⁺ 254.

Halaminol B oxazolidinone (2b): white amorphous solid; [α]_D -20.1° (c 0.008, CH₂Cl₂); ¹H NMR (CDCl₃) δ 5.80, (1H, tdd, *J* = 6, 10 and 17 Hz, H-11), 5.00 (1H, bs, *NH*), 4.98 (1H, brd, *J* = 17 Hz, H-12a), 4.92 (1H, brd, *J* = 10 Hz), H-12b), 4.55 (1H, m, H-3), 3.88 (1H, q, *J* = 7 Hz, H-3), 2.03 (2H, q, *J* = 6 Hz, H-10), 1.72 (1H, m, H-4a), 1.51 (1H, m, H-4b), 1.40–1.25 (10H, m), 1.15 (3H, d, *J* = 6.5 Hz, H-1); ¹³C NMR (CDCl₃) δ 159.1 (s, C-13), 139.1 (t, C-11), 114.2 (d, C-12), 114.2 (d, C-11), 80.2 (d, C-3), 51.1 (d, C-2), 33.8 (t, C-10), 29.1 (t, C-4), 29.3 (t), 29.3 (t), 29.1 (t), 29.0 (t), 28.8 (t), and 16.0 (q, C-1); ESMS *m/z* [M + H]⁺ 226.

Halaminol C oxazolidinone (3b): white amorphous solid; [α]_D -26.7° (c 0.007, CH₂Cl₂); ¹H NMR (CDCl₃) δ 5.54 (1H, m, H-6), 5.34 (1H, m, H-5), 5.26 (1H, bs, *NH*), 4.56 (1H, m, H-3), 3.89 (1H, q, *J* = 7 Hz, H-2), 2.50 (1H, m, H-4a), 2.38 (1H, m, H-4b), 2.03 (2H, m, H-7), 1.40–1.20 (12H, m), 1.18 (3H, d, *J* = 7 Hz, H-1), 0.87 (3H, t, *J* = 7 Hz, H-13); ¹³C NMR (CDCl₃) δ 159.0 (s, C-15), 133.8 (d, C-6), 122.6 (d, C-5), 79.7 (d, C-3), 51.0 (d, C-2), 30.0–28.0 (t, C-8–C-13), 27.5 (t, C-7), 27.2 (t, C-4), 16.0 (q, C-1), and 14.1 (q, C-14); ESMS *m/z* [M + H]⁺ 254.

Preparation of Diacetate Derivatives. A mixture of the halaminols (130 mg) was stirred in a solution of pyridine (3 mL) and acetic anhydride (1 mL) for 24 h at room temperature and then at reflux for 2 h. The reaction mixture was taken up in CH₂Cl₂ (20 mL) and then washed with 1 M NaOH (3 × 10 mL) and saturated NaCl (3 × 10 mL). The organic phase was dried over MgSO₄ and filtered, and the solvent removed, leaving an off-white solid (109 mg). A portion of this product (60 mg) was then separated into halaminol diacetates A (**1c**, 21 mg), B (**2c**, 12 mg), and C (**3c**, 8 mg) by NP HPLC using a mobile phase of 40% EtOAc/hexane.

Diacyl halaminol A (1c): off-white amorphous solid; [α]_D -30° (c 0.003, MeOH); ¹H NMR (CDCl₃) δ 5.79, (1H, tdd, *J* = 7, 11, and 17 Hz, H-13), 5.01 (1H, dd, *J* = 2 and 17 Hz, H-14), 4.91 (1H, dt, *J* = 4 and 7 Hz, H-3), 4.90 (1H, dd, *J* = 2 and 11 Hz, H-14), 4.06 (1H, dq, *J* = 4 and 7 Hz, H-2), 2.04 (3H, s, -OCOCH₃), 2.03 (1H, q, *J* = 7 Hz, H-12), 1.89 (3H, s, -NHCOCH₃), 1.51 (1H, q, *J* = 7 Hz, H-4), 1.40–1.30 (14H, m), 1.09 (3H, d, *J* = 7 Hz, H-1); ¹³C NMR (CDCl₃) δ 172.8 (s, -NHCOCH₃), 172.7 (s, -OCOCH₃), 140.1 (d, C-13), 114.7 (d, C-14), 76.6 (d, C-3), 48.3 (d, C-2), 34.9 (t, C-12), 31.5 (t, C-4), 30.6 (t, C-6), 30.5 (2C, each t, C-7 and C-8), 30.4 (t, C-9), 30.2 (t, C-10), 30.0 (t, C-11), 22.6 (q, -NHCOCH₃), 21.1 (q, -OCOCH₃), and 14.8 (q, C-1); EIMS *m/z* 285 [M]⁺.

Diacyl halaminol B (2c): off-white amorphous solid; [α]_D -20° (c 0.003, MeOH); ¹H NMR (CDCl₃) δ 5.79 (1H, tdd, *J* = 7, 11, and 17 Hz, H-11), 5.01 (1H, dd, *J* = 2 and 17 Hz, H-12), 4.91 (1H, dt, *J* = 4 and 7 Hz, H-3), 4.90 (1H, dd, *J* = 2 and 11 Hz, H-12), 4.05 (1H, dq, *J* = 4 and 7 Hz, H-2), 2.03 (3H, s, -OCOCH₃), 2.03 (2H, q, *J* = 7 Hz, H-10), 1.89 (3H, s, -NHCOCH₃), 1.52 (1H, q, *J* = 7 Hz, H-4), 1.40–1.30 (10H, m), 1.09 (3H, d, *J* = 7 Hz, H-1); ¹³C NMR (CDCl₃) δ 174.2 (s, -OCOCH₃), 174.1 (s, -NHCOCH₃), 141.5 (d, C-11), 116.1 (d, C-12), 78.3 (d, C-3), 49.9 (d, C-2), 36.3 (t, C-10), 32.8 (2C, each t, C-4 and C-9), 32.7 (t, C-8), 22.0 (q, -NHCOCH₃), 20.6 (q, -OCOCH₃), and 16.2 (q, C-1); EIMS *m/z* 257 [M]⁺.

Diacyl halaminol C (3c): off-white amorphous solid; [α]_D -32° (c 0.003, MeOH); ¹H NMR (CDCl₃) δ 5.54 (1H, td, *J* = 7 and 11 Hz, H-6), 5.39 (1H, td, *J* = 7 and 11 Hz, H-5), 4.90 (1H, dt, *J* = 3 and 7 Hz, H-3), 4.07 (1H, dq, *J* = 3 and 7 Hz, H-2), 2.33 (1H, td, *J* = 7 and 14 Hz, H-4), 2.24 (1H, td, *J* = 7 and 14 Hz, H-4), 2.04 (1H, q, *J* = 7 Hz, H-7), 2.03 (3H, s, -OCOCH₃), 1.89 (3H, s, -NHCOCH₃), 1.40–1.30 (10H, m), 1.30 (2H, m, H-13), 1.12 (3H, d, *J* = 7 Hz, H-1); ¹³C NMR (CDCl₃) δ 171.6 (s, -OCOCH₃), 171.5 (s, -NHCOCH₃), 132.9 (d, C-6), 124.1 (d, C-5), 75.8 (d, C-3), 46.9 (d, C-2), 32.0 (t, C-8), 29.7 (t, C-9), 29.6 (t, C-10), 29.4 (2C, each t, C-11 and C-12), 28.6 (t, C-4), 22.7 (t, C-13), 21.6 (q, -NHCOCH₃), 20.0 (q, -OCOCH₃), and 13.9 (q, C-1); EIMS *m/z* 285 [M]⁺.

Preparation of *N*-Acetyl Halaminols. A mixture of the halaminols was acetylated as described above. The diacyl mixture (110 mg) was dissolved in dry methanol (3 mL) with K₂CO₃ (50 mg) and stirred at 40 °C overnight. The resulting cloudy solution was neutralized with 1 M HCl and partitioned between CHCl₃ (10 mL) and H₂O (3 × 5 mL). The organic phase was washed with saturated Na₂CO₃ and H₂O, dried over MgSO₄, and evaporated to dryness to give an acetamide mixture (90 mg). This mixture was separated by NP HPLC using a mobile phase of 70:30 EtOAc/hexane, yielding halaminol A acetamide (**1d**, 35 mg), halaminol B acetamide (**2d**, 12 mg), halaminol C acetamide (**3d**, 11 mg), and halaminol D acetamide (**4**, 17 mg); **1d** and **4** were characterized.

Halaminol A acetamide (1d): white amorphous solid; [α]_D -14.6° (c 0.005, CH₂Cl₂); ¹H NMR (CDCl₃) δ 5.79 (1H, tdd, *J* = 7, 11, and 17 Hz, H-13), 4.90 (1H, dd, *J* = 11 and 2 Hz, H-14a), 5.01 (1H, dd, *J* = 17 and 2 Hz, H-14b), 3.98 (1H, dq, *J* = 3 and 7 Hz, H-2), 3.64 (1H, dt, *J* = 3 and 7 Hz, H-3), 1.89 (3H, s, -NHCOCH₃), 1.49 (2H, q, *J* = 7 Hz, H-4), 1.30–1.40 (14H, m), 1.07 (3H, d, *J* = 7 Hz, H-1); ¹³C NMR (CDCl₃) δ 171.8 (s, -NHCOCH₃), 140.1 (d, C-13), 114.7 (t, C-14), 74.4 (d, C-3), 49.0 (d, C-2), 34.9 (t, C-12), 31.5 (t, C-4), 30.6 (t), 30.5 (t), 30.5 (t), 30.4 (t), 30.2 (t), 30.0 (t), 26.6 (t), 22.6 (-NHCOCH₃), and 14.8 (q, C-1); ESMS *m/z* [M + H]⁺ 270.

Halaminol D acetamide (4): white amorphous solid; [α]_D -20.1° (c 0.017, CH₂Cl₂); ¹H NMR (*d*₅-pyridine) δ 5.57 (m, H-6),

5.45–5.50 (5H, m, H-7, H-9, H-10, H-12, H-15), 5.43 (1H, m, H-16), 5.39 (1H, m, H-13), 4.43 (1H, dq, $J = 3$ and 7 Hz, H-2), 4.01 (1H, dt, $J = 3$ and 7 Hz, H-3), 2.95 (4H, m, H-8 and H-11), 2.88 (2H, t, $J = 5$ Hz, H-14), 2.56 (1H, qd, $J = 7$ and 14 Hz, H-5a), 2.40 (1H, qd, $J = 7$ and 14 Hz, H-5b), 2.06 (2H, m, $J = 7$ Hz, H-17), 2.05 (3H, s, $-\text{NHCOCH}_3$), 1.76 (2H, m, H-4), 1.34 (3H, d, $J = 7$ Hz, H-1), and 0.96 (3H, t, $J = 7$ Hz, H-18); ^{13}C NMR (d_5 -pyridine) δ 170.1 (s, $-\text{NHCOCH}_3$), 132.1 (d, C-16), 130.6 (d, C-6), 128.8 (d, C-7), 128.7 (d, C-9), 128.4 (2C, d, C-10 and C-12), 128.3 (d, C-15), 127.5 (d, C-13), 73.4 (d, C-3), 50.1 (d, C-2), 34.7 (t, C-4), 26.0 (t, C-8), 25.9 (t, C-11), 25.8 (t, C-14), 24.5 (t, C-5), 23.3 (q, $-\text{NHCOCH}_3$), 20.8 (t, C-17), 15.1 (q, C-1), and 14.4 (q, C-18); ESMS m/z $[\text{M} + \text{H}]^+$ 320; HREIMS m/z 319.4873 $[\text{M}]^+$ (calcd for $\text{C}_{19}\text{H}_{31}\text{NO}_2$, 319.4875).

Preparation of MPA Esters of Halaminol A. Halaminol A acetamide (**1d**, 20 mg) was dissolved in dry CH_2Cl_2 (0.2 mL) to which either (*R*)- or (*S*)-MPA (2 equiv, in 0.1 mL of CH_2Cl_2) was added, followed by DCC (1.8 equiv, in 0.1 mL of CH_2Cl_2) and DMAP (1.8 equiv, in 0.1 mL of CH_2Cl_2). The reaction was stirred at room temperature for 30 min and then filtered through a cotton wool plug. The solvent was removed by rotary evaporation and the residue passed through a silica column. The MPA ester (20 mg, 60% yield) eluted with 80:20 CH_2Cl_2 /hexane.

(*R*)-MPA halaminol A acetamide (1e): white amorphous solid; ^1H NMR (CDCl_3) δ 7.50–7.35 (5H, m, Ph), 5.79 (1H, tdd, $J = 6, 10$ and 17 Hz, H-13), 4.97 (1H, brd, $J = 17$ Hz, H-14a), 4.91 (1H, brd, $J = 10$ Hz, H-14b), 4.90 (1H, m, H-3), 4.78 (1H, s, *CH* of MPA), 4.60 (1H, bs, *NH*), 3.98 (1H, q, $J = 7$ Hz, H-2), 3.41 (3H, s, OMe), 2.02 (q, $J = 6$ Hz, H-12), 1.57 (3H, s, NHCOCH_3), 1.56 (1H, m, H-4a), 1.44 (1H, m, H-4b), 1.40–1.15 (14H, m), 0.81 (3H, d, $J = 7$ Hz, H1); ^{13}C NMR (CDCl_3) δ 170.5 (s), 169.0 (s), 139.2 (d, C-13), 129.1 (d, Ph), 128.9 (2C, d, Ph), 127.2 (2C, d, Ph), 114.1 (t, C-14), 82.2 (d), 77.0 (d, C-3), 57.2 (q, OCH_3), 46.9 (d, C-2), 33.8 (t, C-12), 31.4 (t, C-4), 29.5–28.9 (6C, t), 25.8 (t), 23.0 (q, NHCOCH_3), 14.5 (q, C1); ESMS m/z $[\text{M} + \text{H}]^+$ 420, $[\text{M} + \text{Na}]^+$ 442.

(*S*)-MPA halaminol A acetamide (1f): white amorphous solid; ^1H NMR (CDCl_3) δ 7.50–7.30 (5H, m, Ph), 5.79 (1H, tdd, $J = 6, 10$ and 17 Hz, H-13), 5.55 (1H, bs, *NH*), 4.96 (1H, brd, $J = 17$ Hz, H-14a), 4.91 (1H, brd, $J = 10$ Hz, H-14b), 4.83 (1H, m, H-3), 4.79 (1H, s, *CH* of MPA), 4.09 (1H, q, $J = 7$ Hz, H-2), 3.41 (3H, s, OMe), 2.05 (q, $J = 6$ Hz, H-12), 1.79 (3H, s, NHCOCH_3), 1.44 (1H, m, H-4a), 1.38 (1H, m, H-4b), 1.40–0.90 (14H, m), 1.05 (3H, d, $J = 7$ Hz, H-1); ^{13}C NMR (CDCl_3) δ 171.2 (s), 169.6 (s), 139.2 (d, C-13), 128.9 (d, Ph), 128.7 (2C, d, Ph), 127.0 (2C, d, Ph), 114.1 (t, C-14), 82.8 (d), 77.5 (d, C-3), 57.3 (q, OCH_3), 47.8 (d, C-2), 33.8 (t, C-12), 31.2 (t, C-4), 28.8–

29.5 (6C, t), 25.8 (t), 23.1 (q, NHCOCH_3), 14.4 (q, C-1); ESMS m/z $[\text{M} + \text{H}]^+$ 420, $[\text{M} + \text{Na}]^+$ 442.

Antibacterial and Antifungal Assays. Antibacterial and antifungal assays were determined by the paper disk method. Paper disks of diameter 6 mm were impregnated with 0.12 mg of test compound and incubated with the test organisms for 24 h at 28 °C. Settlement assays using larvae of the ascidian *Herdmania curvata* were carried using our standard method; paper disks of diameter 6 mm were impregnated with 50–250 μg of test compound.⁷

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