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Pseudoceratidine, A Marine Natural Product with Antifouling Activity: Synthetic and Biological Studies

James A. Ponasik,^a Susan Conova,^b Denise Kinghorn,^c William A. Kinney,^c Daniel Rittschof,^b and Bruce Ganem^a*

> ^aDepartment of Chemistry, Baker Laboratory, Cornell University Ithaca, New York 14853-1301 U. S. A.

^bDuke University Nicholas School of the Environment, Marine Laboratory, 135 Duke Marine Lab Road, Beaufort, SC 28516-9721 U.S.A.

^CMagainin Pharmaceuticals, 5110 Campus Drive, Plymouth Meeting, PA 19462, U. S. A.

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Abstract: Syntheses of pseudoceratidine and several analogs were developed in order to explore structureactivity relationships responsible for antifouling and antimicrobial activity. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION AND BACKGROUND

Antifouling paints are commonly used to protect ships' hulls, marine pilings, and harbor construction equipment from colonization by microscopic, as well as the propagules of macroscopic, organisms. Besides causing corrosion problems, the attachment of such organisms retards vessels and unnecessarily increases fuel consumption by as much as 40%. The development of effective, low-cost antifouling agents for incorporation into marine coatings has had a major impact on both the recreational and commercial maritime industries, with worldwide demand for such paints now approaching \$2 billion.¹

Copolymers formulated with tributyltin (TBT) methacrylate are the most common antifoulants, used by approximately 80% of the world's commercial shipping fleet. Copper-releasing coatings comprise the bulk of other antifouling paints, after lead and mercury-based coatings were discontinued because of persistent toxicity. When submerged, antifouling paints release toxic compounds at levels (~4 μ g/sq cm of surface per day) that can cause adverse environmental effects.^{2,3} For example, tin-based paints have been linked to the deaths of dolphins, porpoises, and whales in North Atlantic waters since the 1980s. Vessels painted with conventional TBT-based coatings can have a substantial environmental impact while anchored for long periods in an enclosed harbor. Recent concerns about these effects on marine mammals and other organisms have led to a ban on TBT-containing coatings in the United States, Japan, and other countries.

Dedicated to Madeleine M. Joullié in celebration of her distinguished teaching and research career at the University of Pennsylvania.

Many benthic organisms have developed chemical defense systems against predators whose larvae settle and become attached to their prey. Since that process is closely related to biofouling, compounds that block settlement might provide new leads for new antifouling coatings. Studies reported by the Fusetani Biofouling Project in Japan have uncovered a wide spectrum of settlement-inhibiting compounds of marine origin. Among the most potent of these antifouling agents are the spermidine diamide pseudoceratidine 1 derived from a marine sponge (Figure),⁴ as well as dibromotyrosine derivatives such as ceratinamide $A^5 2$ (Figure), ceratinamide B 3, psammaplysin $A^6 4$, ceratinamine⁷ 5, and moloka'iamine⁸ 6.





We^{9a} and others^{9b} have recently described a total synthesis of pseudoceratidine 1, which was reported to inhibit metamorphosis of the barnacle *Balanus amphitrite*. To help delineate structural requirements for this bioactivity, we also synthesized the corresponding N¹ and N⁸ mono-(4,5-dibromo-2-pyrrolyl) amides of spermidine. Naturally-occurring conjugates of various brominated pyrrole carboxylic acids display a wide range of bioactivities, including antimicrobial and immunosuppressive properties,^{10,11,12} and in preliminary assays against a variety of microorganisms, discrimination of the two ends of the polyamine chain was found to be a significant factor in potency.^{9a} Here we expand our earlier study of structure-activity relationships in pseudoceratidine with the synthesis of several additional, closely related mono- and bis-(4,5-dibromo-2pyrrolecarboxamido) residues to test the importance of the pyrrole's substituents and the positively charged secondary amine group in 1. We also describe the results of biological screening indicating the antimicrobial and antifouling potential of these compounds.

RESULTS AND DISCUSSION

Earlier syntheses of 4,5-dibromopyrrole-2-carboxylic acid 8 (Scheme 1) involved bromination of methyl 2-pyrrolecarboxylate followed by deesterification.^{13,14} We developed a more direct route by heating pyrrole-2-carboxylic acid 7 with bromine (2 equiv, 60 °C, HOAc, 1 h), which furnished 8 in 94% yield. The synthesis of pseudoceratidine was completed using the selective acylation procedure of Joshua and Scott.¹⁵

Activation of acid 8 with 1,1'-carbonyldiimidazole (CDI) in DMF followed by treatment with 0.5 equiv of spermidine gave 1 in 73% yield after chromatography. The nonbrominated analog 9 was synthesized from 7 in similar fashion.



To synthesize the N¹ and N⁸ monoacylated analogs of 1 for structure-activity assays, spermidine was first reacted with formaldehyde to afford hexahydropyrimidine 10 (Scheme 2),¹⁶ which was makes possible selective functionalization at the primary nitrogen. Selective acylation of 10 with 8, using CDI in DMF, furnished 11 in 50% yield. Deprotection of the hexahydropyrimidine under Knoevenagel condensation conditions (ethyl hydrogen malonate, pyridine, 78 °C in EtOH) afforded N⁸-(dibromopyrroloyl)-spermidine 12 in 71% yield.



To synthesize N¹-(dibromopyrroloyl)-spermidine, hexahydropyrimidine 10 was first reacted with 2-(t-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON, 0.95 equiv, THF) to generate the carbamateprotected hexahydropyrimidine 13 (Scheme 3) in 43% yield. Deprotection of 13 using ethyl hydrogen malonate as before gave N⁸-BOC-protected spermidine 14, which previously had been described in the patent literature.^{17,18} Selective acylation of 14 with 8 followed by deprotection with HCl afforded N¹-(dibromopyrroloyl)-spermidine 15 in good yield from 10.





To evaluate the importance in biological activity of pseudoceratidine's positive charge, as well as the significance of the diamide backbone's overall length, several mono- and diacyl derivatives of $1,\omega$ -diamines were prepared, as indicated in Scheme 4. Monoacylated analogs 18 and 19 were synthesized by coupling diamines 16 and 17 with 8 using CDI in unoptimized yields of 40% and 23%, respectively. Reaction of 20 and 17 with an excess of 8 afforded the diacylated analogs 21 and 22 each in 40% yield. Compounds 18, 19, 21, and 22 were slightly soluble in CH₂Cl₂, CH₃OH, and DMF, and significant product losses were experienced during chromatographic purification.



Finally, diacylspermine 24 (Scheme 5) was synthesized to examine the effect of an additional positive charge on bioactivity. Coupling of spermine 23 with 8 (2 equiv) afforded 24 in 53% yield.



Examples of other marine-derived spermidine amides, as well as guanidino-polyamines and polyaminesterol conjugates displaying cytotoxic,¹⁹ antimicrobial,²⁰ or antifungal²¹ activity, prompted us to screen 1 and its analogs against a panel of pathogenic microorganisms. Data summarized in columns 1-4 of the Table indicate that all three spermidine analogs prepared in this study (9, 12, 15) were significantly less potent than pseudoceratidine 1. Only the N⁸-monoacylspermidine 12 displayed modest activity against *S. aureus* and *E. coli*. The importance of the pyrrole ring's bromine substituents was underscored by the absence of activity in the nonhalogenated analog 9. Besides being poorly soluble, mono- and diamides of the 4, 7, and 8-carbon diamines were essentially devoid of activity, with one exception. Octanediamine monoamide 19, whose overall structure resembled N⁸-monoacylspermidine 12, displayed marginal antimicrobial behavior against *S. aureus* and *E. coli*. Compound 24, embodying the spermine analog of pseudoceratidine, exhibited broadspectrum antimicrobial activity, with potency comparable to 1. Taken as a whole, the biocidal data in the Table indicate that activity requires the 4,5-dibromopyrrolyl appendage, and is most pronounced with bisamides of polyamines carrying at least one positive charge.

	Antibiotic				Antifouling
Compound	S. aureusa	E. colia	P. aeruginosa ^a	C. albicans ^a	B. amphitrite ^b
1	4	32	128	32	15 (8.0, Ref. 4)
8	n.d. ^c	n.d.	n.d.	<u>n.d.</u>	15
9	>256	>256	>256	>256	n.d.
12	64	128	256	128	inactive
15	>256	256	>256	>256	inactive
18	256	>256	>256	>256	inactive
19	128	128	>256	>256	n.d.
21	>256	>256	>256	>256	0.1
22	>256	>256	>256	>256	n.d.
24	8	32	64	32	45

 Table

 Biological Activity of Pseudoceratidine and Related Polyamines

^a Antibiotic activity reported as minimum inhibitory concentration (MIC) in $\mu g/mL$; ^bAntifouling activity reported as EC₅₀ in $\mu g/mL$; ^cn.d. = not determined.

Several assays based on the surface or swimming behavior of barnacle larvae in flowing systems have been developed to ascertain the effectiveness of antifoulant compounds. Alternatively, by measuring the attachment of larvae to a solid surface, approximate values of EC_{50} (effective concentration corresponding to 50% maximal response) were determined for settlement inhibition (column 5 of the Table). Results with pseudoceratidine 1 were in good agreement with published data. Noteworthy was the finding that 4, 5dibromopyrrole-2-carboxylic acid 8 itself was a potent antifouling agent. The corresponding monoamides of 8 with spermidine and butane-1,4-diamine showed no effect in the barnacle settlement assay. Of special interest, however, was the significant level of antifouling activity displayed by 1,7-diaminoheptane diamide 21. In contrast, spermine diamide 24 was significantly less potent than 21 and less active than the natural product 1. Thus it would appear that the overall length of the diamide chain, and not the net molecular charge, is a powerful determinant of antifouling behavior.

EXPERIMENTAL SECTION

General: Proton-NMR spectra were taken on a Bruker AF-300 or Varian VXR-400S spectrometer. All chemical shifts were reported on the δ scale in parts per million downfield from Me4Si (0.00 ppm). Carbon-13-NMR spectra were taken on a Varian VXR-400S (100 MHz) or Bruker AF-300 (75 MHz) spectrometer. NOTE: Reference quality NMR spectra are available on request. Infrared spectra were taken on a Mattson Galaxy Model infrared spectrometer. Mass spectra were acquired using a Finnigan 3300 mass spectrometer at Cornell or at the University of Illinois Mass Spectrometry Laboratory using a VG ZAB-SE or VG 70-VSE instrument. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected.

Synthesis of 4.5-dibromopyrrole-2-carboxylic acid 8: A solution of bromine (1.47 g, 9.22 mmol) in glacial acetic acid (HOAc, 6.2 mL) was added dropwise at rt to a stirred solution of pyrrole-2-carboxylic acid 7 (0.512 g, 4.6 mmol) in AcOH (25 mL). Upon completion of addition, the solution was heated to 60 °C for 1 h, then cooled to rt and decolorized with Norit. After filtration through Celite, the solvent was removed in vacuo and the resulting solid was crystallized from H₂O/EtOH to afford the known^{13,14} 4,5-dibromopyrrole-2-carboxylic acid 8 (1.17 g, 94%): mp 170 °C (d); ¹H-NMR (300 MHz, DMSO-d₆) δ 10.40 (b, 1 H), 6.82 (d, 1 H, J = 2.7 Hz); ¹³C-NMR (75 MHz, DMSO-d₆) δ 160.4, 125.09, 117.0, 106.8, 98.9; IR (film) 3120, 1695, 1430, 1210, 1180, 760 cm⁻¹; CIMS *m*/z 270 (M⁺, 100%).

Coupling of acid 8 with spermidine to form pseudoceratidine 1: A solution of acid 8 (79 mg, 0.29 mmol) in DMF (0.58 mL) was treated with CDI (48 mg, 0.29 mmol) at rt. The suspension that formed was stirred at rt for 30 min, then treated with spermidine (21 mg, 0.145 mmol) in DMF (0.5 mL), and the reaction stirred at rt for 2 d. After removing the solvent in vacuo, the residue was partitioned between aqueous saturated NaHCO₃ (1.5 mL) and EtOAc (1.5 mL), and the aqueous phase was extracted with EtOAc (3 x 1.5 mL). The combined organic layers were dried (Na₂SO₄), filtered, and concentrated in vacuo. The residue was chromatographed (75:25:3 CH₂Cl₂:MeOH:NH₄OH); to afford 1 (69 mg, 73%) as a solid: mp 62-65 °C; R_f 0.44 (75:25:3 CH₂Cl₂:MeOH:NH₄OH); th-NMR (300 MHz, DMSO-d₆, 380 °K) δ 7.92 (b, 1 H), 7.75 (b, 1 H), 6.86 (s, 1 H), 6.84 (s, 1 H), 3.33 (q, 2 H, J = 4.4 Hz), 3.29 (q, 2 H, J = 6.6 Hz), 2.97 (q, 4 H, J = 7.0 Hz), 1.90 (p, 2 H, J = 6.7 Hz), 1.59-1.73 (m, 4 H); the transformation of the transfo

Acylation of 10 with 8 to form hexahydropyrimidine 11: A solution of acid 8 (80 mg, 0.30 mmol) in DMF (0.6 mL) was treated with CDI (52 mg, 0.32 mmol) at rt. The resulting suspension was stirred at rt. for 30 min, then treated with a solution of hexahydropyrimidine 10 (47 mg, 0.30 mmol) in DMF (0.5 mL) and stirred at rt for 3 d. The solvent was removed in vacuo, and the residue was partitioned between aqueous saturated NaHCO₃ (1.5 mL) and EtOAc (1.5 mL). The aqueous layer was extracted with EtOAc (3 x 1.5 mL), and the combined organic phases were dried (Na₂SO₄), filtered and concentrated. The residue was flash chromatographed (9:1 CH₂Cl₂:NH₃-saturated MeOH) to afford 11 as an oil (60 mg, 50%): Rf 0.22 (9:1 CH₂Cl₂:NH₃-saturated MeOH); ¹H-NMR (300 MHz, DMSO-d₆) δ 8.30 (br, 1 H), 7.90 (br, 1 H), 7.31 (br, 1 H) 7.14 (br, 1 H), 3.48-3.58 (br, 4 H), 2.96 (br, 1 H), 2.79 (br, 3 H), 2.51 (br, 2 H), 1.74-1.76 (br, 6 H).

Conversion of 11 to N^8 -monoacylspermidine 12: A solution of 11 (61 mg, 0.15 mmol) in EtOH (2.5 mL) and pyridine (48 µL, 0.60 mmol) and ethyl hydrogen malonate (82 µL, 0.74 mmol) was stirred at rt for 30 min, then heated at 78 °C for 3 h. After solvent removal in vacuo, the residue was flash chromatographed (7:3:1 CH₂Cl₂:MeOH:NH₄OH) to afford impure 12 contaminated with ethyl hydrogen malonate. To remove the impurity, the residue was dissolved in aqueous HCl (pH 3), extracted with EtOAc (3 x 1.5 mL), and the aqueous layer was freeze dried. The residue was dissolved in H₂O, heated to 100 °C for 1 h, then freeze dried to afford pure 12 (49 mg, 71%): Rf 0.22 (7:3:1 CH₂Cl₂:MeOH:NH₄OH); ¹H-NMR (300 MHz, D₂O) 8.49 (s, 1 H), 7.27 (d, 1 H, J = 1.1 Hz), 6.55 (s, 1 H), 3.13 (t, 2 H, J = 6.7 Hz), 2.82-2.99 (m, 6 H), 1.42-1.57 (m, 4 H); ¹³C-NMR (75 MHz, D₂O, dioxane) 160.9, 126.5, 113.5, 105.5, 98.9, 47.3, 44.4, 38.4, 36.5, 25.8, 23.7, 23.0; HRFAB-MS for C₁₂H₂₁⁷⁹Br⁸¹BrN₄O (M+1), calc 397.0062, found 397.0045.

Conversion of 10 to BOC-hexahydropyrimidine 13: To an ice-cold solution of 10 (0.52 g, 3.3 mmol) in THF (3.5 mL) was added BOC-ON (0.78 g, 3.2 mmol) in THF (13.3 mL), and the solution stirred at 0 °C for 30 min. After concentrating the solvent in vacuo, the residue was dissolved in Et₂O (50 mL) and washed with 5% aqueous NaOH (2 x 10 mL). The organic layer was dried (Na₂SO₄), filtered, and concentrated in vacuo. The residue was flash chromatographed (9:1 CH₂Cl₂:NH₃-saturated MeOH) to afford 13 (0.36 g, 43%) as an oil: R_f 0.39 (9:1 CH₂Cl₂:NH₃-saturated MeOH); ¹H NMR (300 MHz, DMSO-d₆, 380 °K) δ 6.1-6.3 (br, 1 H), 3.27 (s, 2 H), 2.95 (dt, 2 H, J = 5.7, 6.5 Hz), 2.67 (t, 2 H, J = 5.5 Hz), 2.51 (t, 2 H, J = 5.4 Hz), 2.27 (br, 1 H), 2.21 (t, 2 H, J = 6.7 Hz), 1.39-1.53 (m, 13 H); ¹³C NMR (CDCl₃, 100 MHz) 156.1, 78.7, 69.6, 55.0, 52.9, 44.8, 40.4, 28.4, 28.0, 26.5, 24.1; IR (film) 3330, 3220, 2940, 1710, 1175 cm⁻¹; FABMS *m/z* 258 (M+1, 100%).

Preparation of N⁸-BOC-spermidine 14: A solution of hexahydropyrimidine 13 (100 mg, 0.39 mmol) in EtOH (6 mL) containing pyridine (125 μ L, 1.5 mmol) and ethyl hydrogen malonate (215 μ L, 1.9 mmol) was stirred at rt for 30 min, then heated to 78 °C for 4.5 h. The cooled solution was concentrated in vacuo, and the residue flash chromatographed (7:3:1 CH₂Cl₂:MeOH:NH₄OH) to afford impure 14 (177 mg), which was converted to 15 (see below) without further purification.

An analytical sample of 14 was obtained by flash chromatography (7:3:1 CH₂Cl₂:MeOH:NH₄OH): R_f 0.24 (7:3:1 CH₂Cl₂:MeOH: NH4OH); ¹H-NMR (300 MHz, DMSO-d₆, 360 °K) δ 2.95 (t, 2 H, J = 6.8 Hz), 2.73 (br. s, 3 H), 2.68 (t, 2 H, J = 6.7 Hz), 2.59 (t, 2 H, J = 6.8 Hz), 2.53 (t, 2 H, J = 6.6 Hz), 1.53 (q, 2 H, J = 6.8 Hz), 2.54 (t, 2 H, J = 6.7 Hz), 2.59 (t, 2 H, J = 6.8 Hz), 2.54 (t, 2 H, J = 6.7 Hz), 2.59 (t, 2 H, J = 6.8 Hz), 2.54 (t, 2 H, J = 6.7 Hz), 2.59 (t, 2 H, J = 6.8 Hz), 2.54 (t, 2 H, J = 6.7 Hz), 2.59 (t, 2 H, J = 6.8 Hz), 2.55 (t, 2 H, J = 6.6 Hz), 1.53 (q, 2 H, J = 6.8 Hz), 2.54 (t, 2 H, J = 6.7 Hz), 2.54 (t, 2 H, J = 6.8 Hz), 2.55 (t, 2 H, J = 6.6 Hz), 1.55 (t, 2 H, J = 6.8 Hz), 2.54 (t, 2 H, J = 6.8 Hz), 2.54 (t, 2 H, J = 6.8 Hz), 2.55 (t,

6.7 Hz), 1.37-1.47 (m, 13 H); ¹³C-NMR (75 MHz, DMSO-d₆) δ 155.6, 79.2, 59.7, 48.9, 46.9, 31.3, 28.3, 27.4, 26.6; IR (film) 3370, 2930, 1700, 1540, 1370, 1280, 1250, 1175 cm⁻¹.

Conversion of 14 to N¹-monoacylspermidine 15: To a solution of 8 (103 mg, 0.39 mmol) in DMF (1.3 mL) was added CDI (62 mg, 0.39 mmol), and the resulting suspension was stirred at rt for 30 min, then treated with a solution of 14 (94 mg, ca 0.4 mmol) in DMF (1.3 mL). The mixture was stirred at rt for 2 d, then concentrated in vacuo, and the residue partitioned between NaHCO₃ (1.5 mL) and EtOAc (1.5 mL). The aqueous layer was extracted with EtOAc (3 x 1.5 mL) and the combined organic phases were dried (Na₂SO₄), filtered, and concentrated. The residue was chromatographed (9:1 CH₂Cl₂:NH₃-saturated MeOH, then 75:25:3 CH₂Cl₂:MeOH:NH₄OH) to furnish the intermediate N¹-monoacyl-N⁸-BOC-spermidine (71 mg, 36%), which was deprotected by stirring with 1 M HCl in MeOH (0.5 mL) at rt for 3 h. After solvent removal in vacuo, the residue was chromatographed (7:3:1 CH₂Cl₂:MeOH:NH₄OH) to give N¹-monoacylspermidine 15 (55 mg, 83%): R_f 0.19 (7:3:1 CH₂Cl₂:MeOH:NH₄OH); ¹H-NMR (300 MHz, D₂O) 6.61 (s, 1 H), 3.20 (t, 2 H, J = 6.5 Hz), 2.74-2.82 (m, 6 H), 1.70 (q, 2 H, J = 7.4 Hz), 1.48-1.50 (m, 4 H); ¹³C-NMR (75 MHz, D₂O, dioxane) 162.3, 127.7, 114.0, 108.0, 98.1, 47.1, 45.2, 39.0, 36.2, 26.5, 24.6, 23.5; IR (KBr) 3410, 3270, 3050, 2920, 2850, 1635, 1560, 1520, 1410, 1380, 1320, 1240 cm⁻¹; FABMS *m/z* 397 (M+1, 20%).

Coupling of 8 with 1.4-diaminobutane to form monoamide 18: To a solution of 8 (108 mg, 0.40 mmol) in DMF (0.5 mL) was added CDI (101 mg, 0.62 mmol), and after stirring the resulting suspension at rt for 30 min, a solution of diaminobutane 16 (387 mg, 0.44 mmol) in DMF (0.5 mL) was added. The mixture was stirred at rt for 4 d, and solvent was removed in vacuo. The residue was partitioned between saturated NaHCO₃ (3 mL) and EtOAc (1.5 mL). The aqueous layer was extracted with EtOAc (2 x 1.5 mL), and the combined organic layers were dried (Na₂SO₄), filtered, and concentrated. The residue was chromatographed (4:1 CH₂Cl₂:NH₃-saturated MeOH) to give amide 18 (54 mg, 40%): R_f 0.32; ¹H-NMR (300 MHz, DMSO-d₆, 360 °K) δ 7.54 (br, 1 H), 6.73 (s, 1 H), 5.02 (br, 4 H), 3.22 (q, 2 H, J = 4.2 Hz), 2.68 (t, 2 H, J = 6.6 Hz), 1.42-1.60 (m, 4 H); ¹³C-NMR (75 MHz, DMSO-d₆) δ 162.1, 131.52, 112.0, 109.2, 93.9, 48.6, 37.6, 26.8, 26.4; HRFAB-MS for C₉H₁₄⁷⁹Br⁸¹BrN₃O (M+1), calc 339.9483, found 339.9452.

Coupling of 8 with 1.8-diaminooctane to form monoamide 19: Following the procedure for 18, the reaction of acid 8 (50 mg, 0.19 mmol), CDI (43 mg, 0.27 mmol), and diaminooctane 17 (27 mg, 0.19 mmol) afforded monoamide 19 (17 mg, 23%): $R_f 0.28$ (17:3 CH₂Cl₂:NH₃-saturated MeOH); ¹H-NMR (300 MHz, DMSO-d₆, 360 °K) δ 7.54 (br, 1 H), 6.75 (s, 1 H), 4.15 (br, 8 H), 3.20 (q, 2 H, J = 6.4 Hz), 2.65 (t, 2 H, J = 7.0 Hz), 1.39-1.53 (m, 4 H), 1.31 (s, 8 H); ¹³C-NMR (75 MHz, DMSO-d₆) δ 161.3, 130.19, 112.0, 108.1, 94.6, 38.1, 29.5, 28.6, 26.3, 25.9; FABMS *m/z* 396 (M+1, 100%).

<u>Coupling of 8 with 1.7-diaminoheptane to form diamide 21</u>: Following the procedure for 18, the reaction of 8 (52 mg, 0.19 mmol), CDI (32 mg, 0.20 mmol), and diaminoheptane 20 (25 mg, 0.19 mmol) afforded diamide 21 (24 mg, 40%): $R_f 0.57$ (9:1 CH₂Cl₂:NH₃-saturated MeOH); ¹H-NMR (300 MHz, DMSO-d₆) δ 6.75 (s, 2 H), 4.85 (s, 6 H), 3.23 (t, 4 H, J = 7.2 Hz), 1.51 (br, 4 H), 1.30 (br, 6 H); ¹³C-NMR (75 MHz, DMSO-d₆) δ 158.7, 128.3, 112.3, 104.2, 97.7, 38.5, 29.2, 28.5, 26.4; FABMS *m/z* 633 (M+1, 38%).

Coupling of 8 with 1.8-diaminooctane to form diamide 22: Following the procedure for 18, the reaction of 8 (172 mg, 0.64 mmol), CDI (103 mg, 0.64 mmol), and diaminooctane 21 (45 mg, 0.32 mmol) afforded diamide 22 (27 mg, 40%) as a tan solid; mp 165-167 °C; R_f 0.11 (3:97 MeOH:CH₂Cl₂); ¹H-NMR (300 MHz, DMSO-d₆, 360 °K) δ 7.59 (s, 1 H), 6.83 (s, 1 H), 3.21 (q, 2 H, J = 6.8 Hz), 1.52 (m, 2 H, J = 7.1 Hz), 1.32 (s, 4 H); ¹³C-NMR (75 MHz, DMSO-d₆) δ 158.7, 128.3, 112.3, 104.2, 97.7, 38.5, 29.2, 28.7, 26.4; IR (KBr) 3110, 2920, 2860, 1640, 1570, 1530, 1420, 1340, 1250; HRFAB-MS for C₁₈H₂₃⁷⁹Br₂⁸¹Br₂N₄O₂ (M+1), calc 646.8514, found 646.8502.

Coupling of 8 with spermine to form diamide 24: To a solution of 8 (172 mg, 0.64 mmol) in DMF (2.4 mL) was added CDI (103 mg, 0.64 mmol) and the resulting suspension was stirred at rt for 30 min, then treated with spermine 23 (66 mg, 0.33 mmol). The mixture was stirred at rt for 4 d, then concentrated in vacuo. The residue was flash chromatographed (7:3:1 CH₂Cl₂:MeOH:NH₄OH) to afford impure 24 containing acid 8. The mixture was suspended in aqueous HCl (2 mL, pH 3) and filtered to afford spermine 24 as a tan solid (122 mg, 53%): $R_f 0.58$ (7:3:1 CH₂Cl₂:MeOH:NH₄OH); ¹H-NMR (300 MHz, DMSO-d₆) δ 9.01 (s, 2 H), 8.58 (s, 1 H), 7.00 (br. s, 1 H), 3.57 (br. s, 2 H), 2.92 (br. s, 4 H), 1.85 (br. s, 2 H), 1.78 (br. s, 2 H); ¹³C-NMR (75 MHz, DMSO-d₆) δ 159.0, 128.1, 113.1, 104.5, 97.9, 46.0, 44.7, 35.7, 25.9, 22.6; IR (KBr) 3430, 3270, 2950, 2800, 1620, 1580, 1420, 1330, 1250; FABMS *m/z* 705 (M+1, 8%).

Antimicrobial Assays: Minimum inhibitory concentrations (MICs) for *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were determined using a modified version of the National Committee for Clinical Laboratory Standards (NCCLS) approved standard for testing aerobic bacteria in a microdilution broth assay.²² The modification involved using a final volume in each microtiter plate well of 200 μ L rather than 100 μ L. For *Candida albicans* ATCC 90028, MICs were determined using the NCCLS tentative standard for testing antifungal activity of yeasts.²³

Antifouling Assays: A simple settlement assay, developed using the barnacle Balanus amphitrite, was used.²⁴ Working solutions of test compounds were prepared by spreading a thin film of each substance in organic solvent over the bottom of a glass container, evaporating the solvent under vacuum, then mixing the residue with seawater filtered to remove molecules and particulates >100 kDa and aged septically. Triplicate assays on each compound were then repeated on a different batch of larval barnacle cultures to check reproducibility.

Suspensions of three to six-day-old cyprids were pipetted into plastic petri dishes (20-30 cyprids each) containing the test substance at 25, 5, 1, and 0.2 μ g/mL (5 mL final volume). Dishes were incubated at 28 °C for 24 h (12 h light, 12 h dark), and checked for signs of toxicity. The assays were terminated by adding 10% formalin solution. Unattached larvae were rinsed away using deionized water and collected by suction filtration. Attached and free larvae were counted using a microscope. Values of EC₅₀ (effective concentration corresponding to 50% maximal response) for settlement inhibition were estimated by probit analysis²⁵ from counts of a log distribution series of each test compound.

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REFERENCES

- 1. Layman, P. L. Chemical and Engineering News 1995, (May 1), 23.
- 2. Iwata, H.; Tanabe, S.; Mizuno, T.; Tatsukawa, R. Environ. Sci. Tech. 1995, 29, 2959.
- 3. Ohhira, S.; Matsui, H.; Nitta, K. Vet. Human Toxicol. 1996, 38, 206.
- 4. Tsukamoto, S.; Kato, H.; Hiroka, H.; Fusetani, N. Tetrahedron Lett. 1996, 37, 1439.
- 5. Tsukamoto, S.; Kato, H.; Hiroka, H.; Fusetani, N. Tetrahedron 1996, 52, 8181.
- Roll, D. M.; Chang, C. W. J.; Scheuer, P. J.; Gray, G. A.; Shoolery, J. N.; Matsumoto, G. K.; Van Duyne, G. D.; Clardy, J. J. Am. Chem. Soc. 1985, 107, 2916.
- 7. Tsukamoto, S.; Kato, H.; Hiroka, H.; Fusetani, N. J. Org. Chem. 1996, 61, 2936.
- 8. Hamana, M. T.; Scheuer, P. J.; Kelly-Borges, M. J. Org. Chem. 1993, 58, 6565.
- 9. (a) Ponasik, J. A.; Kassab, D. J.; Ganem, B. Tetrahedron Lett. **1996**, 37, 6041; (b) Behrens, C.; Christoffersen, M. W.; Gram, L.; Nielsen, P. H. Bioorg. Med. Chem. Lett. **1997**, 7, 321.
- (a) Forenza, S.; Minale, L.; Riccio, R. J. Chem. Soc. Chem. Commun. 1971, 1129; (b) Gunasekera, S. P.; Cranick, S.; Longley, R. E. J. Nat. Prod. 1989, 52, 757.
- 11. Garcia, E. E.; Benjamin, L. E.; Fryer, R. I. J. Chem. Soc. Chem. Commun. 1973, 78.
- 12. Walker, R. P.; Faulkner, D. J.; Van Engen, D.; Clardy, J. J. Am. Chem. Soc. 1981, 103, 6772.
- 13. Anderson, H. J.; Lee, S.-F. Can. J. Chem. 1965, 43, 409.
- 14. Hodge, P.; Rickards, R. W. J. Chem. Soc. 1965, 459.
- 15. Joshua, A. V.; Scott, J. R. Tetrahedron Lett. 1984, 25, 5725.
- 16. McManis, J. S.; Ganem, B. J. Org. Chem. 1980, 45, 2041.
- 17. Takeuchi, T.; Saino, T.; Yoshida, M.; Takahashi, K.; Nakamura, T.; Umezawa, H. Eur. Pat. Appl. 1986, EP 241,797.
- 18. Takeuchi, T.; Tomioshi, T.; Saino, T.; Takahashi, K.; Nakamura, T. Eur. Pat. Appl. 1989, EP 309,971.
- 19. Schmitz, F. J.; Hollenbeak, K. H.; Prasad, R. S. Tetrahedron Lett. 1979, 3387.
- 20. Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtani, I.; Kusumi, T.; Kakisawa, H. J. Am. Chem. Soc. 1989, 111, 8925.
- (a) Moore, K. S.; Wehrli, S.; Roder, H.; Rogers, M.; Forrest, Jr., J. N.; McCrimmon, D.; Zasloff, M. Proc. Natl. Acad. Sci. U. S. A. 1993, 90, 1354; (b) Wehrli, S. L.; Moore, K. S.; Roder, H.; Durell, S.; Zasloff, M. Steroids 1993, 58, 370.
- "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically," National Committee for Clinical Laboratory Standards, 3rd Ed.; Approved Standard M7-A3, NCCLS, Wayne PA, 1993.
- "Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast," National Committee for Clinical Laboratory Standards; Tentative Standard M27-T, NCCLS, Wayne PA, 1995.
- 24. Rittschof, D.; Clare, A. S.; Gerhart, D. J.; Sister Avelin Mary; Bonaventura, J. Biofouling 1992, 6, 115.
- 25. Lieberman, H. R. Drug. Chem. Toxicol. 1983, 6, 111.