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Article

Phidianidine A and Synthetic Analogues as Naturally Inspired Marine Antifoulants

Christophe Labriere, Vijayaragavan Elumalai, Jannie Staffansson, Gunnar Cervin, Tiffany Le Norcy, Hugo Denardou, Karine Réhel, Lindon W. K. Moodie, Claire Hellio, Henrik Pavia, Jørn H. Hansen, and Johan Svenson*

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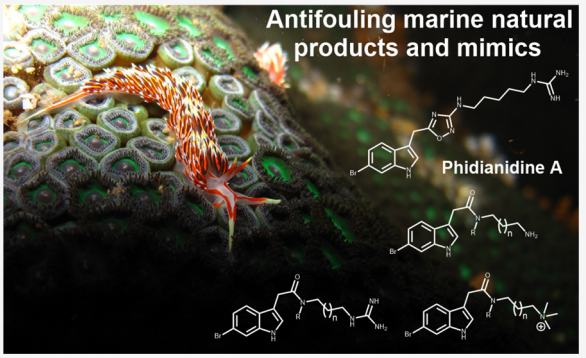
ABSTRACT: Stationary and slow-moving marine organisms regularly employ a natural product chemical defense to prevent being colonized by marine micro- and macroorganisms. While these natural antifoulants can be structurally diverse, they often display highly conserved chemistries and physicochemical properties, suggesting a natural marine antifouling pharmacophore. In our current report, we investigate the marine natural product phidianidine A, which displays several chemical properties found in highly potent marine antifoulants. Phidianidine A and synthetic analogues were screened against the settlement and metamorphosis of *Amphibalanus improvisus* cyprids, and several of the compounds displayed inhibitory activities at low micromolar concentrations with IC_{50} values down to 0.7 μg/mL observed. The settlement study highlights that phidianidine A is a potent natural antifoulant and that the scaffold can be tuned to generate simpler and improved synthetic analogues. The bioactivity is closely linked to the size of the compound and to its basicity. The study also illustrates that active analogues can be prepared in the absence of the natural constrained 1,2,4-oxadiazole ring. A synthetic lead analogue of phidianidine A was incorporated in a coating and included in antifouling field trials, where it was shown that the coating induced potent inhibition of marine bacteria and microalgae settlement.



Marine organisms represent a rich source of novel bioactive natural products with significant chemical diversity and high potency.[1,2] As a result, several drugs and drug leads in clinical trials are of marine origin.[3,4] While slow-moving marine organisms are under a constant threat from predators, sessile organisms also have to deal with the dangers of being colonized and overgrown. In response to these threats, several marine organisms have developed a sophisticated chemical defense to deter predators, colonizers, and competitors.[1,5] Several of these natural products do not directly kill their target organisms but instead function as repellants at low concentrations toward colonizing marine micro- and macroorganisms by acting as antifoulants.[6,7]

Being able to prevent the settlement and fouling of marine structures by marine organisms is complicated, and biofouling countermeasures represent a significant challenge and cost for many marine industries.[8,9] Biofouling is a rapid and naturally occurring process affecting all untreated submerged surfaces which results in unwanted maintenance costs.[8,10] The main challenges are associated with the loss of structural integrity and performance (often increased drag) by the added weight and thickness/rugosity to marine devices and structure.[11] Furthermore, biofouling may promote corrosion, which significantly reduces the effective operation life-span of marine equipment.[12] Methods to provide effective and long-term marine surface protection without general toxicity to nontarget marine organisms are lacking.[13] Hence there is an unmet need for novel and environmentally friendly antifouling solutions. Developing repelling antifouling compounds inspired by Nature represent a promising strategy.[6,13]

A particular group of marine natural products (MNP) with broad antifouling activities are brominated dipeptidic derivatives often isolated from stationary marine organisms.[5] The MNPs in this group share several structural features such as a brominated aromatic moiety, regularly tyrosine or tryptophan, a constrained or heterocyclic center, and often a cationic arm terminated with a guanidine derivative. Ianthelline, initially discovered in the Bahamian sponge *Ianthella ardis*[14] has recently been shown to be a potent natural antifoulant.[15] Ianthelline inhibits the settlement and metamorphosis of barnacle cyprids (IC_{50} = 6 μM) and also displays a pronounced

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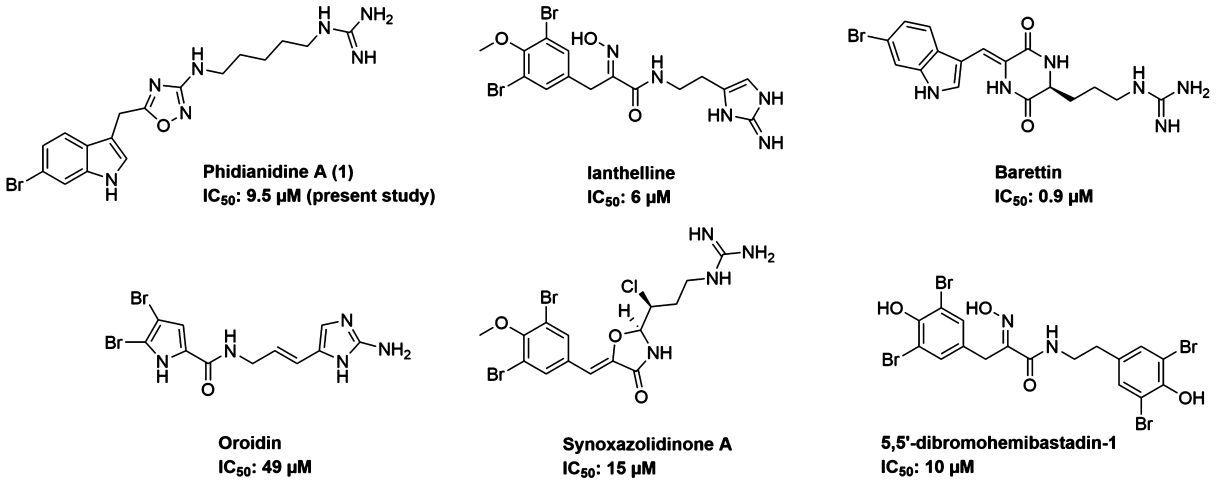


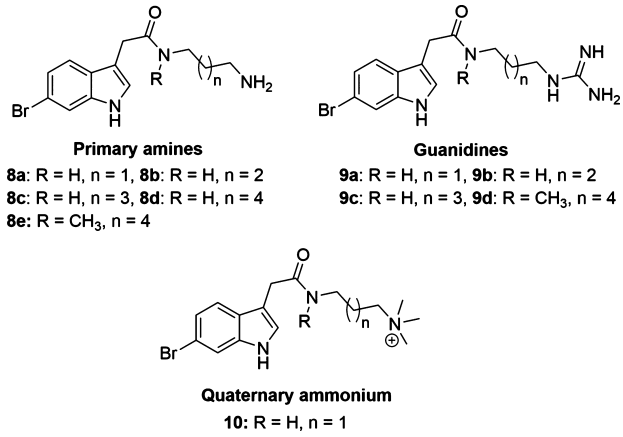
Figure 1. Chemical structure of phidianidine A (**1**) and a selection of natural potent marine barnacle settlement inhibitors with inhibitory concentration (IC_{50}) included.

effect on marine bacteria (minimum inhibitory concentration (MIC) values 0.1−10 μg/mL).[15] The 2,5-diketopiperazine barettin, initially isolated from the sponge *Geodia baretti* in Swedish waters together with structural analogues, prevents the settlement of *Amphibalanus improvisus* at 0.9 μM concentrations.[16] The high potency of barettin has spurred the synthesis of simplified analogues with ranging bioactivities.[17−19] The pyrrole-imidazole oroidin is found in sponges of the *Agelasidae* family where it is considered a likely biogenic precursor to bromoageliferin 1.[20] In addition to being able to inhibit marine bacterial colonization and biofilm formation,[21] oroidin has also been shown in analogy to both ianthelline and barettin, to be a moderate inhibitor of barnacle settlement with a reported IC_{50} value of 49 μM.[22] Furthermore, the synoxazolidinones from the cold-water colonial ascidian *Synoicum pulmonaria*[23−25] and the numerous bastadin analogues commonly isolated from *Ianthella* marine sponges have also been assigned as potent inhibitors of marine micro- and macrofouling.[5,26,27] A selection of potent natural marine inhibitors of barnacle settlement are presented in Figure 1.

Several of the reported marine antifoulants have also been reported with other bioactivities such as antitumoral, anti-inflammatory,[18,28] antibacterial,[21,23] and as inhibitors of acetylcholinesterase.[29,30]

Phidianidine A (**1**), with an uncommon 1,2,4-oxadiazole ring linked to the brominated indole system, is a MNP isolated from the aeolid opisthobranch mollusk *Phidiana militaris*.[31] Compound **1** is structurally and chemically highly analogous to ianthelline, barettin, and the synoxazolidinones and thus displays the structural features often linked to a high antifouling activity. Initial studies of **1** revealed cytotoxic[31] activity, and more recently, **1** and synthetic analogues showed immunosuppressive properties.[32] In addition, **1** was also shown to be a selective inhibitor of the dopamine transporter and a selective, potent ligand and partial agonist of the μ-opioid receptor (versus δ- and κ-opioid receptors).[33] Furthermore, **1** was identified using virtual screening and experimentally verified as a new antagonist of CXCR4 which is a chemokine receptor associated with several diseases like HIV, rheumatoid arthritis, and cancer.[34]

Inspired by the structural resemblance of **1** with several reported highly active antifouling MNPs and the straightforward synthetic tools available for modification, the current study is aimed at evaluation of the antifouling properties of **1** and synthetic analogues. In this report, **1** and a library of 10 synthesized analogues (**8a−e**, **9a−d**, **10**) were prepared and evaluated for the ability to prevent the settlement of *A. improvisus* cyprid. Furthermore, the most promising compound was formulated into a coating and evaluated in field studies for 3 months.



RESULTS AND DISCUSSION

Library Design and Synthesis. The library of compounds was designed to probe the contribution of structure to the activity as previously successfully demonstrated for natural antifoulants.[35−37] With an emphasis on ease of preparation, only **1** was prepared with the 1,2,4-oxadiazole ring motif according to published routes.[38] The rest of the analogues were designed with an amide bond linkage between the brominated indole and the cationic arm. Studies by Lin and Snider have reported a significant loss of activity when the indole bromine is excluded in analogues of **1** and it was decided to keep the 6-bromoindole motif intact.[38] These observations have also been reported for analogues of barettin and the synoxazolidinones when the halogens are removed.[5] Instead focus was placed on the length of the cationic arm and the nature of the terminal cationic group, amine vs guanidine. Two of the compounds were also *N*-methylated and one also contained a quaternary ammonium group. Compound **1** and its analogues **8−10** were all prepared in generally good yields

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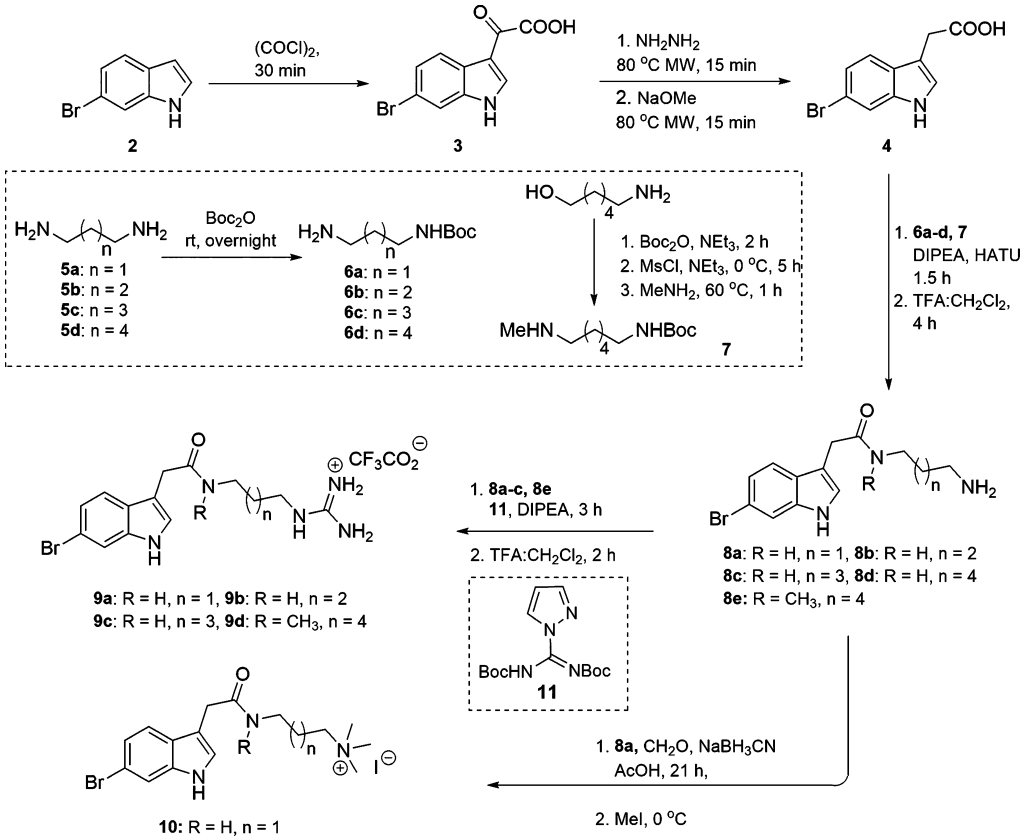

Figure 2. Synthetic outline for compounds **8**−**10**.

with the exception of the *N*-methylated **8e** and **9d** (8 and 26%, respectively). The synthetic scheme employed for preparing compounds **8**−**10** is outlined in Figure 2.

Barnacle Settlement. Several MNPs displaying the functionalities of **1** and analogues have been linked to significant inhibitory potency against the settlement and subsequent metamorphosis of barnacle cyprids. To evaluate the potential of the prepared library as settlement inhibitors, they were tested against *A. improvisus* cyprid larvae. Barnacles represent a major fouling organism,[10] and their tiny footprint and colonization of man-made surfaces results in huge economical implications, leading to multibillion dollar maintenance costs.[39,40] *A. improvisus* originates from the east coast of the American continent but is today spread worldwide via shipping.[41] It represents a major fouling organism and is commonly found on rocks, jetties, and boat hulls where it forms dense colonies on suitable substrata.[39] The extreme tolerance to ranging salinity makes *A. improvisus* a relevant model for studies on both biofouling and osmoregulation.[41]

Most of the evaluated compounds displayed inhibitory effects on the settlement of the barnacle cyprids, and the dose response behavior allowed evaluation of the IC_{50} values for compounds displaying <50% settlement at 5 μg/mL. The degree of inhibition of the compounds and also their potential cyprid toxicity is compiled and compared to the positive control Sea-nine and potent MNPs in Table 1.

Compound **1** displayed an IC_{50} of 4.0 μg/mL and thus it compares well with other established MNPs such as ianthelline,[15] barretin[16] and synoxazolidione A.[5] A 3% mortality of the cyprids was observed at 5 μg/mL which is similar to the negative DMSO control (2.4%) and illustrates that **1** exerts its inhibitory activity via a nontoxic mechanism at the employed concentrations. While several of the simplified synthetic compounds were inactive at 5 μg/mL, compounds **9c** and **9d** displayed potent inhibitory properties with IC_{50} values lower than those observed for **1** at 2.2 and 0.7 μg/mL respectively. Compound **9d** was the most effective inhibitor with activity superior to several potent antifouling MNPs.[6] In addition, the toxicity of **9d** was low as illustrated in Figure 3.

Table 1. Potency and Toxicity of Tested Compounds against the Barnacle *A. improvisus*

compound	metamorphosis (%)	IC_{50} (μg/mL)	toxicity (%)[a]
phidianidine A (**1**)	9	**4.0**	3
8a	66	>5.0	4
8b	77	>5.0	0
8c	58	>5.0	3
8d	72	>5.0	0
8e	58	>5.0	4
9a	67	>5.0	0
9b	73	>5.0	0
9c	27	**2.2**	0
9d	5	**0.7**	6
10	74	>5.0	2
sea-nine[b]	n.d.	0.25	n.d[c]
ianthelline[d]	10	3.0	14
barretin[e]	n.d.	1.0	n.d.
synoxazolidinone A[f]	54	7.4	2

[a]Reported at 5 μg/mL. Toxicity for the negative control DMSO (0.1%, v/v) in filtered seawater was 2.4%. [b]Data from Moodie et al.[36] [c]Not determined. [d]Data from Hanssen et al.[15] [e]Data from Sjögren et al.[16] [f]Data from Trepos et al.[5]

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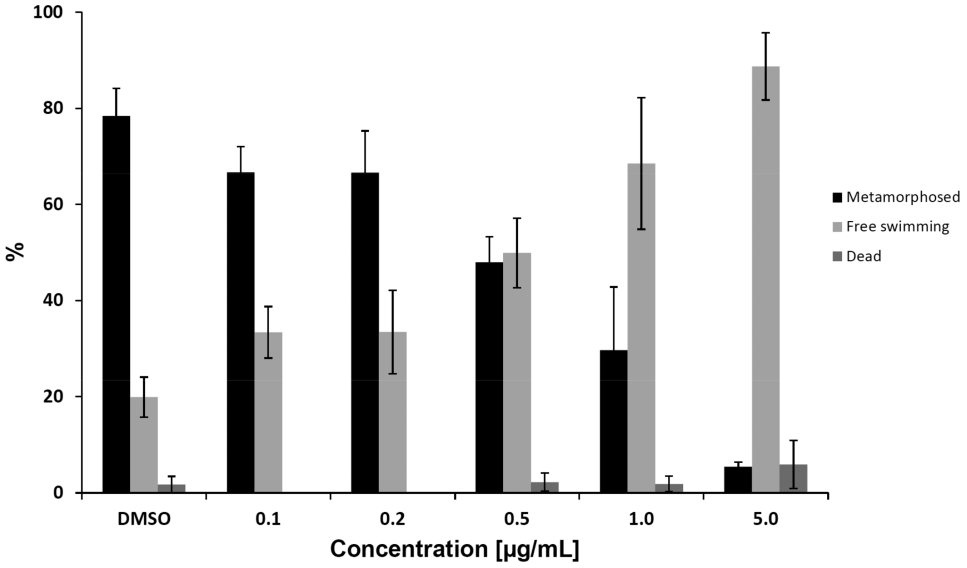

Figure 3. Dose response analysis (0.1−5.0 μg/mL) of **9d** on the settlement inhibition of *A. improvisus* cyprid larvae presented as percentages of settled (black columns), free swimming (light gray columns), and dead cyprids (dark gray columns) and given as means ± standard error (n = 4). DMSO (0.1%, v/v) in filtered seawater was used as the negative control.

The current library was composed of compounds probing the effects of basicity and hydrogen bonding capacity of the cationic arm[42] and also the distance between the brominated indole and the cationic charge. Several structure activity relationship studies on these specific functionalities and properties have been described for related MNPs[17,43] and natural antifoulants.[35,37,44] In Nature, most of these MNPs resemble a dipeptide in size and this library contain compounds within that size span with a number of shorter analogues. The active compounds **1**, **9c**, and **9d** share several structural features. They all bear guanidine functionalities, and they all represent the longest compounds in the libraries with either five or six methylenes in the alkane linker. **9d** is the longest compound in the study and also the most active one. The shorter guanidine bearing compounds **9a** and **9b** are inactive as are the primary amines **8a**−**8e**, indicating that the increased basicity and hydrogen bonding capacity is essential for activity.[42] This correlates well with related antifouling MNPs which commonly display a guanidine or a guanidine derivative.[15,16,22] Compound **10**, which displays a cationic quaternary ammonium group, is inactive. The *N*-methylated **8e** represents an amine version of the highly active **9d** but is inactive and illustrates how potential benefits arising from the *N*-methylation is not sufficient in the absence of a guanidine functionality. Like all the compounds in the library, the indole motifs of **9c** and **9d** are linked with the cationic arm with an amide bond as opposed to the natural 1,2,4-oxadiazole ring found in **1**. Both **9c** and **9d** display higher antifouling activity than **1**, which illustrates that the 1,2,4-oxadiazole ring is not essential for activity if sufficient linker length and basicity is provided. This is an important observation and many studies on analogues of **1** in medically relevant screens have incorporated the synthesis of the 1,2,4-oxadiazole ring system into the library of screened compounds.[32,33] Our studies suggest that significantly simplified analogues can be used to yield bioactive compounds with even improved activity. Collectively, the barnacle cyprid screen highlights that **1** is a potent natural antifoulant and that the scaffold can be used to generate simpler and improved synthetic analogues. The activity is linked to the size of the compound and to the basicity of the cationic group and the study demonstrates that active analogues can be prepared in the absence of the constrained 1,2,4-oxadiazole ring.

Coating Development. Given the high and repelling activity of **9d**, it was decided to evaluate the ability of the compound to inhibit fouling in field immersion experiments. Such an experiment would also establish the general antifouling potential of the compound as the natural biofouling consortium contains microfoulers such as marine bacteria, micro- and macroalgae, and other potential macrofoulers in addition to barnacles. Many studies are published on antifouling experiments performed in laboratory bioassay settings but it is often highly speculative to conclude about the repelling potential of a compound or coating without access to data from field experiments.[45] In addition, the search for new environmentally friendly, cost-effective, and legally acceptable ways of preventing growth of marine organisms on marine materials is urgent, as reviewed by Ciriminna[46] and Kyei.[47] To generate a coating suited for field studies, a previously developed biodegradable poly(ε-caprolactone-*co*-δ-valerolactone) (80:20) polymer for antifouling applications was prepared and solubilized in xylene.[48] Compound **9d** was added to the polymer, and 200 μm thick polymer coatings were prepared on PVC panels, which were submerged for 84 days at a depth of 1 m in the port of Kernével in Lorient, France during the summer. The panels were regularly investigated for growth during the 84-day study, and the growth on the panels during the period is shown in Figure 4.

After 84 days the panels were removed from the water and the growth was assessed using confocal laser scanning microscopy (CLSM). On a macroscopic level no obvious differences between the different panels can be observed. No pronounced "edge effects", often observed during evaluation novel antifouling coatings, were seen on the treated panels either.[49] Growth was observed on the treated panels after 28 days, while the control panels displayed growth after 14 days. This small difference however disappears with time. At the end of the experiments, the panels are seemingly covered to a

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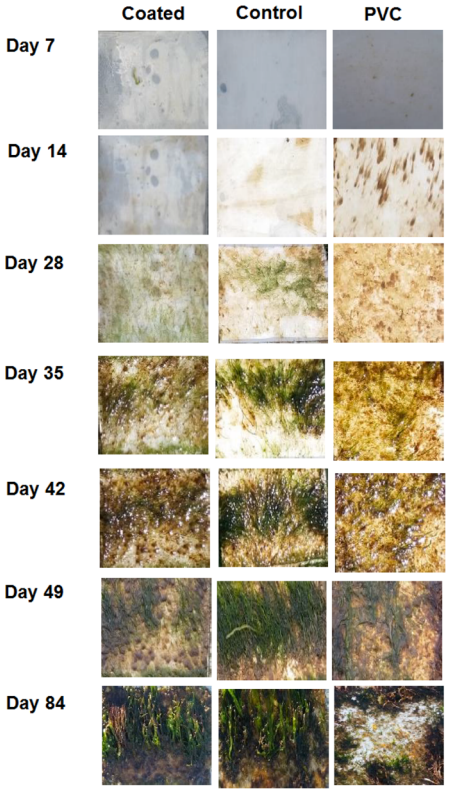

Figure 4. Results from the field trials illustrating the buildup of biofouling communities. Left column: **9d** containing coating applied to PVC panel, center column: negative control coating without **9d** on PVC panel, right column: control PVC panel without any coating.

similar extent with a layer of biofouling organisms, the bulk of which appear to be chlorophycea (*Ulva intestinalis*) and phaeophycea macroalgae. It is not surprising to find such organisms on the panels as the formulation did not contain any photosynthesis inhibitors such as commercial herbicides. No barnacles were observed on any of the panels making assessments of the potential repelling effect of **9d** on the barnacle settlement inconclusive. The reproductive period of *A. improvisus* is mainly from May to September and the presence of free swimming cyprids occurs during this period.[41] The reason for the general low settlement of barnacles during the experimental period is thus likely coupled with unexpected seasonal or local variability in free swimming cyprids and not due to specific repelling effects from the different panels and their coatings. CLSM analysis of the biofilms however revealed differences between the coatings as displayed in Figure 5.

The different staining methods employed allowed quantification of both marine bacteria and microalgae. Clear differences are seen between the coating incorporation **9d** and the control coating.[50] The coating incorporating **9d** displays both lower coverage and biovolumes of bacteria (3-fold) and microalgae (6-fold), suggesting that **9d** also displays potent antifouling activities against marine microfoulers. The recorded reduction in biofilm establishment was observed at lower concentrations (in the coating) than those described for dibromohemibastadin-1 *in vitro*[50] and also correlates well with *in situ* studies up to 35 days.[51] Also in those recent studies, a more pronounced effect was seen on microalgal biofilm formation than for bacterial biofilms.[51] Our current study shows that this effect persists for up to 84 days for **9d**. Related compounds such as the synaxozolidiones[5] and ianthelline[15] are powerful inhibitors of the microfouling biofilm with IC_{50} values down to 10 ng/mL reported, which is 2 orders of magnitude lower than the concentration of **9d** in the coating. This illustrates how this class of compounds are often highly repelling against both micro- and macrofoulers. In parallel, while studies are ongoing on evaluating extracts of marine organisms to generate effective marine antifouling coatings,[49] a focus on single compounds of known concentrations provides a more extensive understanding of the potency and mode of action. Being able to generate functional and environmentally benign coatings based on natural products or simplified synthetic mimics has been heralded as a promising way toward a new generation of antifouling coating technologies,[47] and our work sheds additional light on the performance of these compounds and materials *in vitro* and in the field.

CONCLUSIONS

The antifouling potential of the MNP phidianidine A (**1**) has been established, and our study illustrates that **1** is a potent, nontoxic inhibitor of barnacle cyprid metamorphosis. Structure−activity studies employing simplified synthetic analogues illustrate that the antifouling activity against *A. improvisus* barnacles can be tuned, and optimized analogues can readily be prepared while excluding the 1,2,4-oxadiazole ring. A promising antifouling lead was identified (**9d**), and it was incorporated into an antifouling coating and evaluated in field trials for 84 days. A strong reduction in the settlement of microfouling organisms was shown, illustrating that the lead analogue also is a powerful inhibitor of microfouling. The effect on barnacle settlement in the field was inconclusive because of low amounts of naturally free-swimming barnacle cyprids during the field experiments. The current study sheds additional light on the structural motif of MNPs governing slow-moving or sessile marine organism protection against colonizing organisms and illustrates how these compounds can be significantly structurally simplified with maintained or even improved bioactivities.

EXPERIMENTAL SECTION

IR spectra were obtained on an Agilent Technologies Cary 630 FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature at a frequency of 400 and 101 MHz, respectively on a Bruker spectrometer. The chemical shifts are reported in ppm and are referenced to the relevant solvent peak: $CDCl_3$ at δ_H 7.26 and δ_C 77.16; CD_3OD at δ_H 3.31 and δ_C 49.0; $(CD_3)_2SO$ at δ_H 2.50 and δ_C 39.5 ppm. High-resolution mass spectra (HRMS) were recorded using MeOH solution on LTQ Orbitrap XL in either positive or negative electrospray ionization (ESI) modes. TLC was performed on Merck silica gel 60 F254 plates, using UV light at 254 nm, and PMA staining followed by heating for detection. Flash column chromatography was performed by using the indicated solvent system and silica gel (40−63 μm). Microwave reactions were performed in 20 mL vials using a Discover SP from CEM using an Anton Parr Monowave 300 instrument. All reagents and solvents were purchased commercially and used directly without any further purification.

Synthesis. *Phidianidine A (1).* A sample of phidianidine (**1**) was prepared for biological testing according to literature procedures.[38,52] The substituted 1,2,4-oxadiazole ring of **1** was prepared via the route described for the synthesis of phidianidine by Manzo et al.[52] from *N*-Boc-1-amino-5-[(E)-2-hydroxyguanidino]-pentane and 6-bromo-3-indoleacetic acid ethyl ester and was subsequently guanidylated with *N,N′*-di-Boc-1*H*-pyrazole-1-carboxamidine according to Lin and Snider.[38] Compound **1** was prepared in 28% yield (2 steps) with spectral data matching those previously reported. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.56−7.51 (m, 1H), 7.48−7.42 (m, 1H), 7.24

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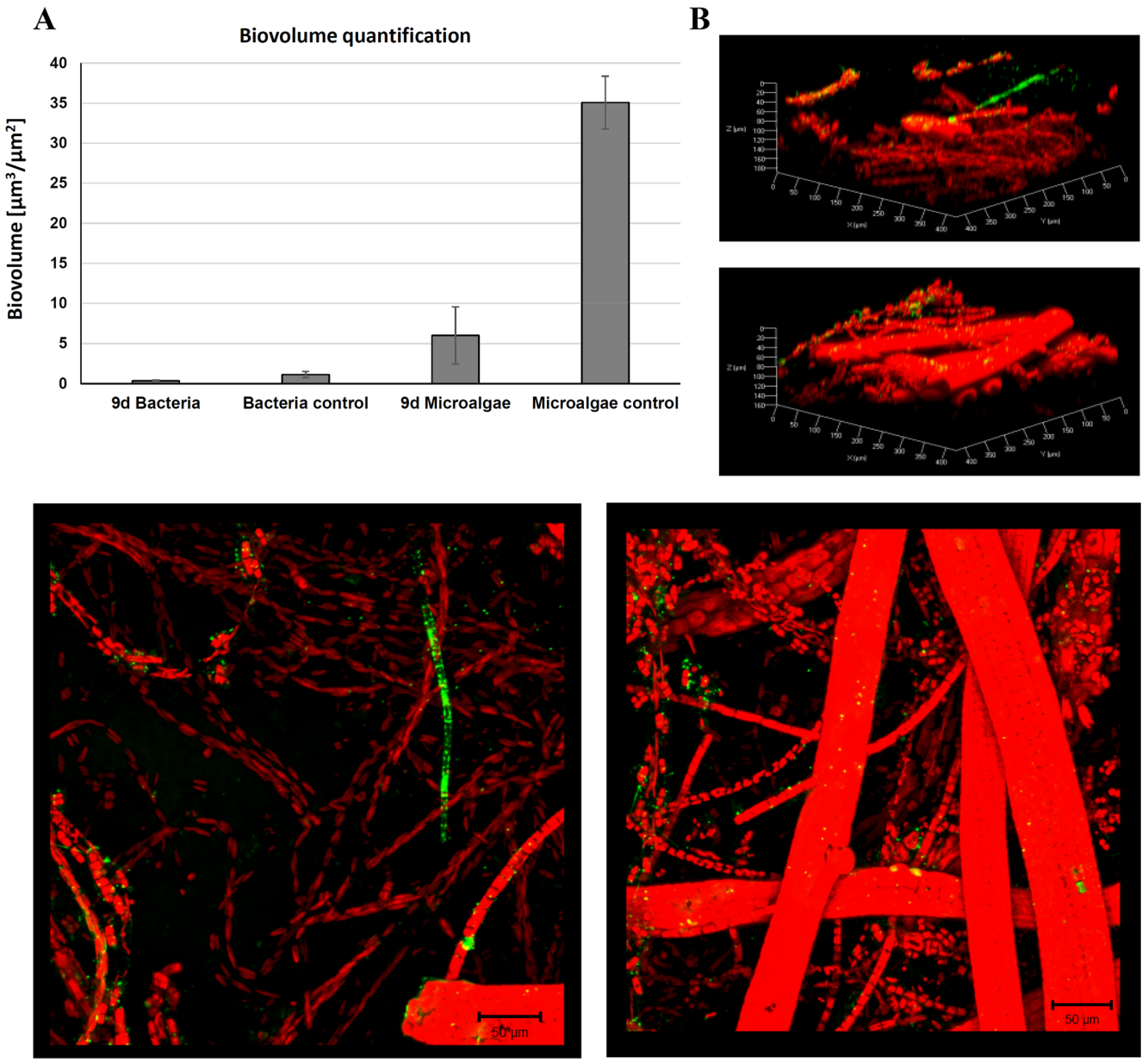

Figure 5. Upper row: quantification of the biovolumes ($\mu m^3/\mu m^2$) of adhered bacteria and microalgae after 84 days immersion (A) and CLSM images used to assess the volume of the biofilms (B). Bottom row illustrates a top view of the formed biofilm on the surfaces coated with **9d** (left) and the control varnish (right).

(t, J = 0.9 Hz, 1H), 7.13 (dd, J = 8.5, 1.8 Hz, 1H), 4.20 (d, J = 0.8 Hz, 2H), 3.19−3.09 (m, 4H), 1.66−1.54 (m, 4H), 1.48−1.37 (m, 2H).^{13}C NMR (101 MHz, Methanol-d_4) δ 178.98, 170.10, 158.63, 138.89, 127.16, 125.71, 123.23, 120.82, 116.18, 115.30, 108.68, 43.77, 42.35, 29.74, 29.51, 24.90, 23.90. FTMS m/z 420.1131 $[M + H]^+$ (calcd for $C_{17}H_{23}{}^{79}BrN_7O^+$, 420.1142).

*2-(6-Bromo-1H-indol-3-yl)-2-oxoacetic Acid (**3**).* To a solution of 6-bromoindole (1.00 g, 5.1 mmol, 1.0 equiv) in diethyl ether (20 mL) was added oxalyl chloride (0.89 mL, 10.2 mmol, 2.0 equiv). The solution was stirred at room temperature (rt) for 45 min. Water (0.5 mL, 27.8 mmol, 5.0 equiv) was carefully added to the solution, followed by diethyl ether (50 mL). The resulting mixture was stirred for 30 min, filtered, washed with diethyl ether, and dried under vacuum to afford **3** as a yellow powder (1.05 g, 77%). IR (cm^{-1}). 3167, 3146, 1722, 1626, 1410, 1227, 1134, 888, 795, 754, 683. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.66 (s, 1H), 12.52 (s, 1H), 8.49 (d, J = 3.3 Hz, 1H), 8.30 (d, J = 2.0 Hz, 1H), 7.52 (d, J = 8.6 Hz, 1H), 7.42 (dd, J = 8.6, 2.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 180.6, 164.8, 139, 135.5, 127.4, 126.3, 123.2, 115.5, 114.8, 111.8.

FTMS m/z 265.9455 $[M-H]^-$ (calcd for $C_{10}H_5{}^{79}BrNO_3{}^-$, 265.9458).

*2-(6-Bromo-1H-indol-3-yl)acetic Acid (**4**).* To a microwave-vial equipped with a stir bar were added **3** (1.00 g, 3.74 mmol, 1.0 equiv), 2-ethoxyethanol (10 mL), and hydrazine (0.91 mL, 18.73 mmol, 5.0 equiv). The vial was sealed and stirred for 15 min at 80 °C under microwave irradiation. Sodium methoxide (1.84 g, 34.1 mmol, 10.0 equiv) was then added, and the reaction mixture resealed and stirred for 1 h at 160 °C under microwave irradiation before being quenched with 4 M HCl (20 mL), H_2O (20 mL), and EtOAc (50 mL); extracted with EtOAc (2 × 20 mL); dried over Na_2SO_4; and the solvent removed under reduced pressure. The crude product was purified by silica gel column chromatography [(EtOAc + 1% AcOH:n-heptane, 4:6)] to afford **4** as a pale yellow solid (0.76 g, 80%). R_f = 0.125 [(EtOAc:n-heptane, 1:1)]. IR (cm^{-1}). 3417, 2933, 2895, 2117, 1693, 1402, 1223, 1097, 866, 803, 773, 695. ^{1}H NMR

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(400 MHz, Methanol-d_4) δ 7.68 (d, J = 1.8 Hz, 1H), 7.26 (d, J = 8.6 Hz, 1H), 7.20−7.16 (m, 2H), 3.69 (s, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 176.1, 136.6, 130.4, 126.2, 125.2, 122.2, 113.9, 113, 108.8, 31.8. FTMS m/z 251.9670 $[M\text{-}H]^-$ (calcd for $C_{10}H_7{}^{79}BrNO_2^-$, 251.9666).

*Monoboc Protected Diamines (**6a−d**).* Monoboc protected amines **6a−d** were prepared using standard protocols.[53] The synthesis of compound **6d** is provided as a representative example.

*tert-Butyl-(6-aminohexyl)carbamate (**6d**).* To a solution of 1,5-diaminohexane (5.00 g, 43.03 mmol, 4.0 equiv) in dioxane:H_2O (9:1, 50 mL) was added dropwise a solution of di-*tert*-butyl dicarbonate (2.47 mL, 10.76 mmol, 1.0 equiv) in dioxane: H_2O (9:1, 50 mL) over 3 h. The resulting mixture was stirred at rt overnight. The solvent was removed under reduced pressure and the residue was suspended in H_2O (50 mL) and filtered. The filtrate was then extracted with CH_2Cl_2 (3 × 20 mL), washed with H_2O, dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure to afford **6d** as a white oil (6.6 g, 71%). IR (cm^{-1}). 3361, 2977, 2929, 2858, 1696, 1529, 1369, 1253, 1171. ^{1}H NMR (400 MHz, Chloroform-d) δ 4.73 (s, 1H), 3.02 (q, J = 6.8 Hz, 2H), 2.62−2.57 (m, 2H), 1.45−1.28 (m, 13H), 1.28−1.21 (m, 4H), 1.04 (s, 2H). ^{13}C NMR (101 MHz, Chloroform-d) δ 156, 78.8, 42.1, 40.4, 30, 28.4, 26.7, 26.6, 26.5. FTMS m/z 217.1924 $[M + H]^+$ (calcd for $C_{11}H_{25}N_2O_2^+$, 217.1911).

*tert-Butyl-(6-(methylamino)hexyl)carbamate (**7**).* To a solution of 6-hydroxy-1-hexylamine (2.80 g, 24 mmol) in CH_2Cl_2 (50 mL), was added di-*tert*-butyl dicarbonate (5.2 g, 4.7 mmol). Triethylamine (3.7 mL) was added dropwise, and the reaction stirred at rt for 2 h. The reaction mixture was then washed with H_2O (20 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The solvent was evaporated to afford *N*-(*tert*-butoxycarbonyl)-6-hydroxy-1-hexylamine (4.95 g, 95%) as pale-white solid, which was used without further purification.[1]H NMR (400 MHz, Chloroform-d) δ 4.56 (s, 1H), 3.61 (td, J = 6.5, 5.3 Hz, 2H), 3.09 (q, J = 6.7 Hz, 2H), 1.77 (t, J = 5.5 Hz, 1H), 1.59−1.51 (m, 2H), 1.50−1.44 (m, 2H), 1.42 (s, 9H), 1.35 (ddd, J = 14.9, 9.0, 3.9 Hz, 4H).^{13}C NMR (101 MHz, Chloroform-d) δ 156.1, 79.1, 62.6, 46.0, 40.4, 32.6, 31.2, 30.1, 28.4, 26.4, 25.3. To a 0 °C solution of *N*-(*tert*-butoxycarbonyl)-6-hydroxy-1-hexylamine (2.0 g, 9.2 mmol, 1.0 equiv) in CH_2Cl_2 (20 mL) was added methane sulfonyl chloride (0.85 mL, 9.3 mmol, 1.1 equiv). Triethylamine (1.5 mL, 9.4 mmol, 1.2 equiv) was added dropwise to the 0 °C solution, and then the reaction was stirred at rt for 5 h. The solvents were removed under reduced pressure to afford *N*-(*tert*-butoxycarbonyl)-6-((methanesulfonyl)oxy)hexylamine (2.65 g, 97%) as white crystalline solid, which was used without further purification.[1]H NMR (400 MHz, $CDCl_3$) δ 4.18 (t, J = 6.5 Hz, 2H), 3.07 (t, J = 7.0 Hz, 2H), 2.97 (s, 3H), 1.78−1.65 (m, 2H), 1.51−1.43 (m, 2H), 1.40 (s, 9H), 1.39−1.27 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.0, 79.1, 70.0, 37.3, 29.9, 29.0, 28.4, 26.2, 25.1. To a solution of *N*-(*tert*-butoxycarbonyl)-6-((methanesulfonyl)oxy)hexylamine (2.6 g, 8.97 mmol, 1.0 equiv) in dioxane (20 mL) was slowly added aqueous methylamine (20 mL, 40%, 60 equiv). The mixture was stirred at 60 °C for 1 h and monitored by TLC (CH_2Cl_2/MeOH/NH_3 aq., 9.0:0.9:0.1, PMA stain), concentrated under reduced pressure to afford the product **7** as a light beige solid (1.2 g, 58%), which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 (t, J = 7.0 Hz, 2H), 2.97−2.85 (m, 3H), 2.65 (t, J = 5.5 Hz, 2H), 1.83 (q, J = 7.6 Hz, 2H), 1.41 (s, 15H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.1, 79.1, 49.2, 38.6, 32.9, 29.7, 29.3, 28.4, 26.1, 26.0, 25.7. FTMS m/z 231.2061 $[M + H]^+$ (calcd for $C_{12}H_{27}N_2O_2^+$, 231.2067).

*N-(3-Aminopropyl)-2-(6-bromo-1H-indol-3-yl)acetamide (**8a**).* To a solution of **4** (1.00 g, 3.94 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) were added DIPEA (0.75 mL, 4.33 mmol, 1.1 equiv) and HATU (1.497 g, 3.94 mmol, 1.0 equiv). The mixture was stirred at rt for 30 min. A solution **6a** (0.755 g, 4.33 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) was then added, and the resulting mixture was stirred at rt for 90 min. A solution of CH_2Cl_2:TFA (1:1, 20 mL) was directly added to the reaction mixture and stirred for 3 h at rt. The dark brown solution was quenched with H_2O (20 mL) and the pH adjusted to 12 with 4 M NaOH (35 mL), extracted with CH_2Cl_2 (2 × 20 mL), dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The crude product was purified by silica gel column chromatography [(CH_2Cl_2: 100 to MeOH: 100)] to afford **8a** as a light yellow oil (0.89 g, 73%). R_f = 0.03 [(MeOH: 100)]. IR (cm^{-1}). 3253, 2929, 2869, 2117, 1640, 1529, 1454, 1343, 1227, 1108, 884, 795. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.71 (d, J = 1.9 Hz, 1H), 7.27 (d, J = 8.6 Hz, 1H), 7.21−7.16 (m, 2H), 3.59 (s, 2H), 3.23 (t, J = 6.7 Hz, 2H), 2.57 (t, J = 6.9 Hz, 2H), 1.60 (p, J = 6.8 Hz, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.7, 136.7, 130.3, 126.5, 125.3, 122, 114, 113.1, 109.4, 39.6, 37.8, 33.8, 33.3. FTMS m/z 310.0567 $[M + H]^+$ (calcd for $C_{13}H_{17}{}^{79}BrN_3O^+$, 310.0550).

*N-(4-Aminobutyl)-2-(6-bromo-1H-indol-3-yl)acetamide (**8b**).* To a solution of **4** (0.500 g, 2 mmol, 1.0 equiv) in CH_2Cl_2 (7 mL) were added DIPEA (0.38 mL, 2.2 mmol, 1.1 equiv) and HATU (0.760 g, 2 mmol, 1 equiv). The mixture was stirred at rt for 30 min. A solution of **6b** (0.414 g, 2 mmol, 1.1 equiv) in CH_2Cl_2 (7 mL) was then added, and the resulting mixture was stirred at room rt for 90 min. A solution of CH_2Cl_2:TFA (1:1, 20 mL) was directly added to the reaction mixture and it was stirred for 3 h at rt. The dark brown solution was quenched with H_2O (20 mL) and the pH adjusted to 12 with 4 M NaOH (35 mL), extracted with CH_2Cl_2 (2 × 20 mL), dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The crude product was purified by silica gel column [(CH_2Cl_2: 100 to MeOH: 100)] to afford **8b** as a light yellow oily solid (0.28 g, 43%). R_f = 0.03 [(MeOH: 100)]. IR (cm^{-1}). 3249, 2925, 2858, 2113, 1640, 1529, 1454, 1343, 1227, 1108, 884, 795. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.72 (d, J = 1.8 Hz, 1H), 7.27 (d, J = 8.6 Hz, 1H), 7.21−7.16 (m, 2H), 3.59 (s, 2H), 3.17 (t, J = 6.7 Hz, 2H), 2.57 (t, J = 7.0 Hz, 2H), 1.51−1.35 (m, 4H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.5, 136.7, 130.3, 126.5, 125.2, 122.1, 114, 113.1, 109.5, 42.1, 40.3, 33.9, 30.9, 27.7. FTMS m/z 324.0713 $[M + H]^+$ (calcd for $C_{14}H_{19}{}^{79}BrN_3O^+$, 324.0706).

*N-(5-Aminopentyl)-2-(6-bromo-1H-indol-3-yl)acetamide (**8c**).* To a solution of **4** (1.00 g, 3.94 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) were added DIPEA (0.75 mL, 4.33 mmol, 1.1 equiv) and HATU (1.497 g, 3.94 mmol, 1 equiv). The mixture was stirred at rt for 30 min. A solution of **6c** (0.876 g, 4.33 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) was then added, and the resulting mixture was stirred at rt for 90 min. A solution of CH_2Cl_2:TFA (1:1, 20 mL) was directly added to the reaction mixture and it was stirred for 3 h at rt. The dark brown solution was quenched with H_2O (20 mL) and the pH adjusted to 12 with 4 M NaOH (35 mL), extracted with CH_2Cl_2 (2 × 20 mL), dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The crude product was purified by silica gel column chromatography [(CH_2Cl_2: 100 to MeOH: 100)] to afford **8c** as a light yellow oily solid (0.92 g, 69%). R_f = 0.03 [(MeOH: 100)]. IR (cm^{-1}). 3242, 2929, 2858, 2125, 1640, 1529, 1439, 1343, 1227, 1108, 884, 795. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.72 (d, J = 1.8 Hz, 1H), 7.27 (d, J = 8.6 Hz, 1H), 7.22−7.15 (m, 2H), 3.59 (s, 2H), 3.17 (t, J = 6.9 Hz, 2H), 2.53 (t, J = 7.0 Hz, 2H), 1.52−1.34 (m, 4H), 1.30−1.18 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.4, 136.7, 130.3, 126.5, 125.2, 122.1, 114, 113.1, 109.5, 42.4, 40.4, 33.9, 33.4, 30.2, 25.1. FTMS m/z 338.0878 $[M + H]^+$ (calcd for $C_{15}H_{21}{}^{79}BrN_3O^+$, 338.0863).

*N-(6-Aminohexyl)-2-(6-bromo-1H-indol-3-yl)acetamide (**8d**).* To a solution of **4** (1.00 g, 3.94 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) were added DIPEA (0.75 mL, 4.33 mmol, 1.1 equiv) and HATU (1.497 g, 3.94 mmol, 1.0 equiv). The mixture was stirred at rt for 30 min. A solution of **6d** (0.937 g, 4.33 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) was then added, and the resulting mixture was stirred at rt for 90 min. A solution of CH_2Cl_2:TFA (1:1, 20 mL) was directly added to the reaction mixture and was stirred for 3 h at rt. The dark brown solution was quenched with H_2O (20 mL) and the pH adjusted to 12 with 4 M NaOH (35 mL), extracted with CH_2Cl_2 (2 × 20 mL), dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The crude product was purified by silica gel column chromatography [(CH_2Cl_2: 100 to MeOH: 100)] to afford **8d** as a light yellow oily solid (0.23 g, 33%). R_f = 0.03 [(MeOH: 100)]. IR (cm^{-1}). 3238, 2929, 2858, 2180, 1644, 1529, 1439, 1361, 1227, 1108, 884, 795. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.71 (d, J = 1.9 Hz, 1H), 7.27 (d, J = 8.6 Hz, 1H), 7.21−7.16 (m, 2H), 3.59 (s, 2H), 3.16

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(t, J = 6.9 Hz, 2H), 2.58 (t, J = 7.2 Hz, 2H), 1.48−1.36 (m, 4H), 1.31−1.22 (m, 4H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.4, 136.8, 130.3, 126.5, 125.2, 122.1, 114, 113.1, 109.5, 42.3, 40.4, 38.9, 33.9, 33.4, 30.3, 27.6. FTMS m/z 352.1027 $[M + H]^+$ (calcd for $C_{16}H_{23}{}^{79}BrN_3O^+$, 352.1019).

*N-(6-Aminohexyl)-2-(6-bromo-1H-indol-3-yl)-N-methylacetamide (**8e**).* To a solution of **4** (0.560 g, 2.20 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) were added DIPEA (0.45 mL, 2.44 mmol, 1.1 equiv) and HATU (0.900 g, 2.20 mmol, 1 equiv). The mixture was stirred at rt for 30 min. A solution of **7** (0.550 g, 2.44 mmol, 1.1 equiv) in CH_2Cl_2 (10 mL) was then added, and the resulting mixture was stirred at rt for overnight. A solution of CH_2Cl_2:TFA (1:1, 20 mL) was directly added to the reaction mixture and was stirred for 3 h at rt. The dark brown solution was quenched with H_2O (20 mL) and the pH adjusted to 12 with 4 M NaOH (35 mL), extracted with CH_2Cl_2 (2 × 20 mL), dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The crude product was purified by silica gel column chromatography [(CH_2Cl_2: 100 to MeOH: 100)] to afford **8e** as a pale-yellow oil (61 mg, 8%) ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.68−7.56 (m, 1H), 7.15 (ddt, J = 8.9, 6.2, 2.8 Hz, 1H), 7.11−6.99 (m, 2H), 3.80−3.59 (m, 2H), 3.31−3.21 (m, 2H), 3.03−2.77 (m, 3H), 2.56−2.41 (m, 2H), 1.36−1.21 (m, 4H), 1.14−0.94 (m, 4H). ^{13}C NMR (101 MHz, Methanol-d_4) δ: 172.4, 135.4, 129.0, 124.5, 123.9, 121.0, 112.61, 111.7, 107.5, 50.2, 47.4, 47.2, 47.0, 40.6, 35.0, 32.5, 31.1, 27.7, 26.6, 26.0. FTMS m/z 366.1176 $[M + H]^+$ (calcd for $C_{17}H_{25}{}^{79}BrN_3O^+$, 366.1176).

*Amino-((3-(2-(6-bromo-1H-indol-3-yl)acetamido)propyl)amino)-methaniminium 2,2,2-trifluoroacetate (**9a**).* To a solution of **8a** (90 mg, 0.3 mmol, 1.0 equiv) and *N,N′*-Di-Boc-1*H*-pyrazole-1-carboxamidine (0.18 g, 0.59 mmol, 2.0 equiv) in THF (5 mL) was added DIPEA (0.1 mL, 0.59 mmol, 2.0 equiv). The mixture was stirred at rt for 3 h, quenched with H_2O (10 mL), extracted with diethyl ether (2 × 10 mL), washed with sat. $NaHCO_3$ and brine (10 mL), dried over Na_2SO_4, and concentrated. The crude product was purified on silica gel column chromatography [(EtOAc:Hept-n, 6:4)] to give the intermediate as a transparent oil (0.15 g, 91%). R_f = 0.21 [(EtOAc:Hept-n, 4:1)]. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.83 (broad s, 1H), 7.31−7.14 (m, 3H), 3.64 (s, 2H), 3.39−3.29 (m, 4H, solvent), 3.22 (broad s, 2H), 1.60−1.36 (m, 20H). ^{13}C NMR (101 MHz, Methanol-d4) δ 174.4, 164.4, 157.9, 153.9, 136.6, 130.2, 126.4, 125.2, 122.2, 114, 113.1, 109.5, 84.3, 80.4, 38.8, 37.3, 34.1, 30.1, 28.6, 28.2. FTMS m/z 552.1878 $[M + H]^+$ (calcd for $C_{24}H_{35}{}^{79}BrN_5O_5^+$, 552.1816).

The intermediate (0.15 g, 0.27 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (5 mL) and a solution of TFA:CH_2Cl_2 (1:1, 10 mL) was added. The reaction mixture was stirred at rt for 2 h, quenched with H_2O, extracted with CH_2Cl_2 (2 × 10 mL), washed with H_2O (2 × 10 mL), and the solvent was removed under reduced pressure to afford **9a** as an orange oil (95 mg, 75%). ^{1}H NMR (400 MHz, Methanol-d4) δ 7.78 (d, J = 1.9 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.30−7.22 (m, 2H), 3.72−3.67 (m, 2H), 3.30 (t, J = 6.8 Hz, 2H), 3.17 (t, J = 7.0 Hz, 2H), 1.78 (p, J = 6.9 Hz, 2H). ^{13}C NMR (101 MHz, Methanol-d4) δ 173.8, 173.7, 135.4, 128.9, 125.2, 123.9, 120. 7, 112.8, 111.8, 107.9, 47.5, 47.2, 47.0, 38.5, 36.2, 32.5, 32.4, 28.5. FTMS m/z 352.0813 $[M + H]^+$ (calcd for $C_{14}H_{19}{}^{79}BrN_5O^+$, 352.0767).

*Amino-((4-(2-(6-bromo-1H-indol-3-yl)acetamido)butyl)amino)-methaniminium 2,2,2-trifluoroacetate (**9b**).* To a solution of **8b** (0.19 g, 0.59 mmol, 1.0 equiv) and *N,N′*-Di-Boc-1*H*-pyrazole-1-carboxamidine (0.37 g, 1.17 mmol, 2.0 equiv) in THF (7 mL) was added DIPEA (0.21 mL, 1.17 mmol, 2.0 equiv). The mixture was stirred at rt for 3 h, quenched with H_2O (10 mL), extracted with diethyl ether (2 × 10 mL), washed with sat. $NaHCO_3$ and brine (10 mL), dried over Na_2SO_4, and concentrated. The crude product was purified on silica gel column chromatography [(EtOAc:Hept-n, 6:4)] to give the intermediate as a light white powder (0.29 g, 86%). R_f = 0.19 [(EtOAc:*n*-heptane, 4:1)]. IR (cm^{-1}). 3413, 3286, 2933, 2866, 2516, 2445, 2378, 2154, 1722, 1629, 1581, 1458, 1369, 1287, 1153, 1052, 1030, 877, 803, 739. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.71 (d, J = 1.8 Hz, 1H), 7.26 (d, J = 8.6 Hz, 1H), 7.21−7.16 (m, 2H), 3.60 (s, 2H), 3.31−3.25 (m, 2H), 3.21 (t, J = 6.6, 5.8 Hz, 2H), 1.52 (s, 13H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.5, 164.5, 157.6, 154.2, 136.7, 130.3, 126.5, 125.3, 122.1, 114, 113.1, 109.4, 84.4, 80.3, 41.4, 40.2, 33.9, 28.6, 28.2, 27.6, 27.5. FTMS m/z 566.2031 $[M + H]^+$ (calcd for $C_{25}H_{37}{}^{79}BrN_5O_5^+$, 566.1973).

The intermediate (0.24 g, 0.42 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (5 mL) and a solution of TFA:CH_2Cl_2 (1:1, 10 mL) was added. The reaction mixture was stirred at rt for 2 h, quenched with H_2O, extracted with CH_2Cl_2 (2 × 10 mL), washed with H_2O (2 × 10 mL), and the solvent was removed under reduced pressure to afford **9b** as a green-orange oil (167 mg, 83%). IR (cm^{-1}). 3286, 3181, 2944, 2125, 1633, 1566, 1436, 1436, 1182, 1134, 1026, 888, 840, 803, 724. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.70 (d, J = 1.8 Hz, 1H), 7.27 (d, J = 8.6 Hz, 1H), 7.20−7.16 (m, 2H), 3.60 (s, 2H), 3.19 (t, J = 6.1 Hz, 2H), 3.10 (t, J = 6.9 Hz, 2H), 1.53−1.47 (m, 4H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.7, 158.5, 136.7, 130.2, 126.5, 125.2, 122, 114.1, 113.1, 109.4, 42, 39.8, 33.8, 27.5, 27.0. FTMS m/z 366.0947 $[M + H]^+$ (calcd for $C_{15}H_{21}{}^{79}BrN_5O^+$, 366.0924).

*Amino-((5-(2-(6-bromo-1H-indol-3-yl)acetamido)pentyl)amino)-methaniminium 2,2,2-trifluoroacetate (**9c**).* To a solution of **8c** (0.40 g, 1.19 mmol, 1.0 equiv) and *N,N′*-Di-Boc-1*H*-pyrazole-1-carboxamidine (0.74 g, 2.39 mmol, 2.0 equiv) in THF (8 mL) was added DIPEA (0.42 mL, 2.39 mmol, 2.0 equiv). The mixture was stirred at rt for 3 h, quenched with H_2O (10 mL), extracted with diethyl ether (2 × 10 mL), washed with sat. $NaHCO_3$ and brine (10 mL), dried over Na_2SO_4, and concentrated. The crude product was purified on silica gel column chromatography [(EtOAc:*n*-heptane, 6:4)] to give the intermediate as a light white powder (0.576 g, 83%). R_f = 0.26 [(EtOAc:*n*-heptane, 4:1)]. IR (cm^{-1}). 3413, 3288, 2981, 2936, 1722, 1618, 1577, 1417, 1369, 1328, 1134, 1058, 1030, 884, 799. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.71 (d, J = 1.8 Hz, 1H), 7.27 (d, J = 8.9 Hz, 1H), 7.21−7.17 (m, 2H), 3.60 (s, 2H), 3.27 (t, J = 7.2 Hz, 2H), 3.18 (t, J = 6.8 Hz, 2H), 1.58−1.49 (m, 13H), 1.46 (s, 9H), 1.33−1.26 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.5, 164.6, 157.5, 154.2, 136.7, 130.3, 126.4, 125.3, 122.1, 114, 113.1, 109.5, 84.4, 80.3, 41.7, 40.3, 33.9, 30, 29.7, 28.6, 28.2, 25.1. FTMS m/z 580.2158 $[M + H]^+$ (calcd for $C_{26}H_{39}{}^{79}BrN_5O_5^+$, 580.2129).

The intermediate (0.55 g, 0.95 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (5 mL) and a solution of TFA:CH_2Cl_2 (1:1, 10 mL) was added. The reaction mixture was stirred at rt for 2 h, quenched with H_2O, extracted with CH_2Cl_2 (2 × 10 mL), washed with H_2O (2 × 10 mL) and the solvent was removed under reduced pressure to afford **9c** as a yellow-orange oil (112 mg, 24%). IR (cm^{-1}). 3283, 3176, 2940, 2125, 1633, 1566, 1462, 1436, 1182, 1138, 1028, 888, 840, 803, 724. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.70 (d, J = 1.9 Hz, 1H), 7.27 (d, J = 8.6 Hz, 1H), 7.20−7.16 (m, 2H), 3.60 (s, 2H), 3.17 (t, J = 6.9 Hz, 2H), 3.04 (t, J = 7.1 Hz, 2H), 1.54−1.44 (m, 4H), 1.32−1.25 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 174.6, 158.5, 136.7, 130.2, 126.5, 125.2, 122.1, 114.1, 113.1, 109.4, 42.3, 40.2, 33.9, 29.9, 29.3, 24.8. FTMS m/z 380.1092 $[M + H]^+$ (calcd for $C_{16}H_{23}{}^{79}BrN_5O^+$, 380.1080).

*2-(6-Bromo-1H-indol-3-yl)-N-(6-guanidinohexyl)-N-methylacetamide Trifluoroacetate (**9d**).* To a solution of crude **8e** (0.049 g, 0.14 mmol, 1.0 equiv) and *N,N′*-Di-Boc-1*H*-pyrazole-1-carboxamidine (0.087 g, 0.28 mmol, 2.0 equiv) in THF (10 mL) was added DIPEA (0.05 mL, 0.28 mmol, 2.0 equiv). The mixture was stirred at rt for 3 h, quenched with H_2O (20 mL), extracted with diethyl ether (3 × 20 mL), washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The diboc-intermediate compound (confirmed by HRMS) was used without further purification and dissolved in TFA:CH_2Cl_2 (1:1, 10 mL), and the reaction mixture was stirred at room temperature for 2 h, quenched with H_2O, extracted with CH_2Cl_2 (2 × 10 mL), washed with H_2O (2 × 10 mL), and the solvent was removed under reduced pressure to afford **9d** as brown oil (19 mg, 26%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.63 (dd, J = 4.9, 1.9 Hz, 1H), 7.18 (dd, J = 8.6, 3.1 Hz, 1H), 7.13−7.01 (m, 2H), 3.72 (d, J = 0.9 Hz, 2H), 3.30 (td, J = 7.4, 2.5 Hz, 2H), 3.02−2.96 (m, 2H), 2.97 (d, J = 11.6 Hz, 3H), 1.49−1.28 (m, 4H), 1.23−1.02 (m, 4H). ^{13}C NMR (101 MHz, Methanol-d_4) δ: 172.5, 157.2, 135.4, 128.9, 124.7, 123.9, 121.0, 112.6, 111.7, 107.5, 54.4, 40.9, 34.9, 32.5, 31.1,

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28.3, 26.4, 25.7. FTMS m/z 408.1406 $[M + H]^+$ (calcd for $C_{18}H_{27}{}^{79}BrN_5O^+$, 408.1393).

*3-(2-(6-Bromo-1H-indol-3-yl)acetamido)-N,N,N-trimethylpropan-1-aminium Iodide (**10**).* To a 0 °C solution of **8a** (100 mg, 0.32 mmol, 1.0 equiv) and glacial acetic acid (72 μL, 1.28 mmol, 4.0 equiv) in MeOH (5 mL) under argon atmosphere, was added sodium cyanoborohydride (40 mg, 0.64 mmol, 2.0 equiv). A solution of formaldehyde (21 μL, 0.38 mmol, 2.4 equiv) in MeOH (2 mL) was carefully added dropwise over 10 min, and then stirred at rt for 21 h, quenched with Na_2CO_3 (2 M) until pH 8−9 and concentrated under reduced pressure. The residue was taken up in $CHCl_3$, washed with brine and dried over Na_2SO_4, filtered, and concentrated to obtain the dimethylated product. To a 0 °C solution of the dimethylated product (80 mg, 0.24 mmol) in $CHCl_3$ (1 mL) was added iodomethane (0.05 mL, 0.72 mmol, 10 equiv) in portions (× 3). The precipitate was isolated, coevaporated with MeOH (× 3) to afford the product **10** as a light yellow oil (81 mg, 53%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.80 (d, J = 1.9 Hz, 1H), 7.39−7.28 (m, 2H), 7.23 (dd, J = 8.7, 1.9 Hz, 1H), 3.68 (s, 2H), 3.29 (t, J = 6.4 Hz, 2H), 3.25−3.18 (m, 2H), 3.00 (s, 9H), 2.01−1.86 (m, 2H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 173.6, 135.4, 128.9, 125. 6, 124.0, 120.8, 113.0, 111. 8, 108.1, 64.3, 52.3, 47.7, 47. 5, 47.2, 47.0, 35.7, 32.0. FTMS m/z 352.1027 $[M]^+$ (calcd for $C_{16}H_{23}{}^{79}BrN_5O^+$, 352.1019).

Barnacle Settlement. The barnacle cyprids were reared in a laboratory rearing system, and their settlement was evaluated according to the methods of Berntsson at Tjärnö Marine Biological Laboratory.[54] In brief, a total of 18−22 freshly molted *A. improvisus* cyprids were added to nontreated polystyrene Petri dishes (48 mm, Nunc #150340) containing 10 mL of filtered (0.2 μm) seawater (freshly collected from a depth of 45 m from the Kosterfjord (Sweden), filtered through 20 μm filter and diluted with fresh water to 25 psu prior to use), and the compounds were serially diluted with DMSO (10 μL added) to yield the desired concentration series. The dishes were incubated at ambient temperature (20−25 °C) for 5 days, and at the end of the experiment, the number of metamorphosed juvenile barnacles, as well as live and dead cyprids, was assessed under a dissection microscope. The concentration of a compound leading to 50% inhibition of the settlement (metamorphosed cyprids) compared to the control was reported as the IC_{50} value. Dead cyprids are not included in the IC_{50} value. Dishes with 10 μL of DMSO added served as negative control. Sea-nine was used as positive control, and each test concentration was replicated four times (n = 4).

Coating Development. A biodegradable poly(ε-caprolactone-*co*-δ-valerolactone) (80:20) polymer was prepared according to previously published methodology[48] and solubilized in xylene (mixture of isomers) (1:1 w/w). The compound was dissolved in MeOH (1 M final concentration) and added to polymer solution to yield a final compound concentration of 0.1 mg/L. The solution was vortexed for 1 min and subsequently ultrasonicated (3 × 15 min) to yield a homogeneous coating solution which was applied at a thickness of 200 μm to PVC panels (10 × 10 cm^2) using a film applicator. The coating was allowed to dry for 24 h at ambient temperature before further analyses and studies.

Field Studies. The coated and uncoated reference PVC panels as well as control panels coated with polymer in the absence of compound were mounted on structures and immersed at a depth of 1 m deep in the port of Kernével in Lorient (France, 47°42′20.78″ N, 3°23′40.68″ E). Each type of panel was included in triplicate in the field study. The immersion was carried out in spring at an average water temperature of 16 °C and a salinity of 29 mg/mL. The panels were regularly controlled and photographed to document and assess the settlement of organisms. After day 84, the panels were retrieved, and the extent and type of colonization was analyzed and quantified.

Prior to quantification of growth, the panels were gently rinsed to remove loosely adhered material and the adhesion of cells was observed with confocal laser scanning microscopy (Zeiss, LSM 710) by using a 40× oil immersion objective for bacteria and 20× air objective for diatoms. Adhered bacteria were observed with Syto9 nucleic acid stain (5 μM, $\lambda_{excitation}$ = 488 nm, $\lambda_{emission}$ = 498−540 nm). Adhered diatoms were observed by their fluorescence ($\lambda_{excitation}$ = 633 nm, $\lambda_{emission}$ = 638−720 nm). The extent of growth, biovolume, and overlap percentage was determined with a JAVA program (Universite' de Bretagne-sud, Lorient, France).[50,51]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00881.

^{1}H NMR and ^{13}C NMR data for all new synthetic compounds and synthesized phidianidine A together with 2D NMR data for **8a**, **9c**, and **10** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Johan Svenson − *Department of Chemistry, Chemical Synthesis and Analysis Group, UiT The Arctic University of Norway, N-9037 Tromsø, Norway; Department of Chemistry, Biomaterial & Textile, RISE Research Institutes of Sweden, 501 15 Borås, Sweden;* orcid.org/0000-0002-4729-9359; Phone: +64 27 272 1876; Email: Johan.svenson@cawthron.org.nz

Authors

Christophe Labriere − *Department of Chemistry, Chemical Synthesis and Analysis Group, UiT The Arctic University of Norway, N-9037 Tromsø, Norway*

Vijayaragavan Elumalai − *Department of Chemistry, Chemical Synthesis and Analysis Group, UiT The Arctic University of Norway, N-9037 Tromsø, Norway*

Jannie Staffansson − *Department of Chemistry, Chemical Synthesis and Analysis Group, UiT The Arctic University of Norway, N-9037 Tromsø, Norway*

Gunnar Cervin − *Department of Marine Sciences, Tjärnö Marine Laboratory, University of Gothenburg, SE-452 96 Strömstad, Sweden*

Tiffany Le Norcy − *Univ. Bretagne-Sud, EA 3884, LBCM, IUEM, F-56100 Lorient, France*

Hugo Denardou − *Department of Chemistry, Chemical Synthesis and Analysis Group, UiT The Arctic University of Norway, N-9037 Tromsø, Norway*

Karine Réhel − *Univ. Bretagne-Sud, EA 3884, LBCM, IUEM, F-56100 Lorient, France*

Lindon W. K. Moodie − *Department of Medicinal Chemistry and Uppsala Antibiotic Centre, Biomedical Centre, Uppsala University, 75123 Uppsala, Sweden;* orcid.org/0000-0002-9500-4535

Claire Hellio − *Univ. Brest, Laboratoire des Sciences de l'Environnement MARin (LEMAR), CNRS, IRD, IFREMER, Brest 29285, France*

Henrik Pavia − *Department of Marine Sciences, Tjärnö Marine Laboratory, University of Gothenburg, SE-452 96 Strömstad, Sweden*

Jørn H. Hansen − *Department of Chemistry, Chemical Synthesis and Analysis Group, UiT The Arctic University of Norway, N-9037 Tromsø, Norway;* orcid.org/0000-0002-3888-5217

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.0c00881

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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