JOURNAL OF NATURAL PRODUCTS

Article

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Exploring the Sponge Consortium *Plakortis symbiotica–Xestospongia deweerdtae* as a Potential Source of Antimicrobial Compounds and Probing the Pharmacophore for Antituberculosis Activity of Smenothiazole A by Diverted Total Synthesis

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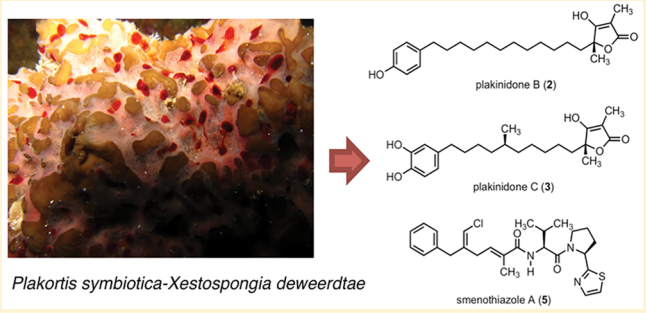
Supporting Information

ABSTRACT: Fractionation of the bioactive $CHCl_3$–MeOH (1:1) extracts obtained from two collections of the sponge consortium *Plakortis symbiotica–Xestospongia deweerdtae* from Puerto Rico provided two new plakinidone analogues, designated as plakinidone B (**2**) and plakinidone C (**3**), as well as the known plakinidone (**1**), plakortolide F (**4**), and smenothiazole A (**5**). The structures of **1**–**5** were characterized on the basis of 1D and 2D NMR spectroscopic, IR, UV, and HRMS analysis. The absolute configurations of plakinidones **2** and **3** were established through chemical correlation methods, VCD/ECD experiments, and spectroscopic data comparisons. When assayed in vitro against *Mycobacterium tuberculosis* $H_{37}Rv$, none of the plakinidones **1**–**3** displayed significant activity, whereas smenothiazole A (**5**) was the most active compound, exhibiting an MIC value of 4.1 μg/mL. Synthesis and subsequent biological screening of **8**, a dechlorinated version of smenothiazole A, revealed that the chlorine atom in **5** is indispensable for anti-TB activity.



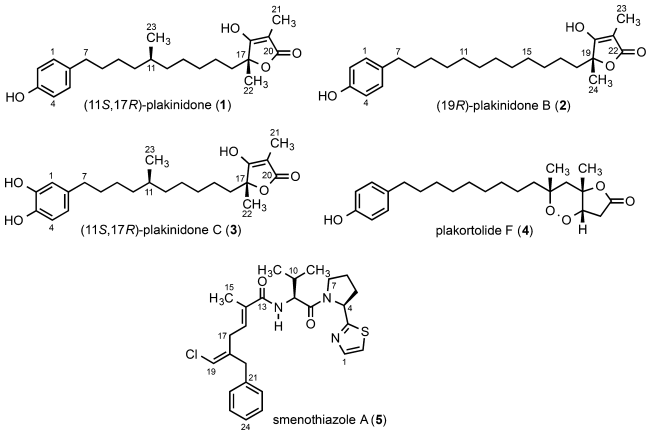
For almost a decade, two groups of marine natural products chemists have gradually scrutinized the chemical composition of the symbiotic two-sponge association *Plakortis halichondrioides–Xestospongia deweerdtae*.[1,2] These still-ongoing investigations have led to the discovery of a variety of structurally interesting cyclic polyketide peroxides with diverse biological activities.[1−3] Remarkably, by using a combination of molecular and morphological data, Vicente and co-workers recently demonstrated that the *Plakortis* species involved in this unusual epizoic symbiosis is not *P. halichondrioides* but a new sponge species, namely, *Plakortis symbiotica*.[4]

During a 2006 expeditionary voyage to Mona Island, located in a strait between the Dominican Republic and Puerto Rico, samples of a sponge consortium constituted by *Plakortis symbiotica* (Vicente, Zea & Hill, 2016) and *Xestospongia deweerdtae* (Lehnert & van Soest, 1999) specimens were collected by members of our research team at a depth of 90–100 ft. The extract of a small portion of the consortium containing mainly *P. symbiotica* was found to be strongly toxic to a series of human tumor cells and two pathogenic microbes (*Mycobacterium tuberculosis* and *Plasmodium falciparum*); thus the sponge was targeted for chemical analysis and extracted with $CHCl_3$–MeOH. After solvent removal, the dark gum obtained was suspended in H_2O and extracted exhaustively with *n*-hexane and $CHCl_3$. In 2010, we reported on the results of our chemical investigation of the *n*-hexane extract after further fractionation, which led to the discovery of two strongly biologically active five-membered-ring polyketide endoperoxides, each containing a conjugated triene along an unbranched C_{16} alkyl side chain.[2b] As part of our ongoing effort to identify novel metabolites from this sponge–sponge association, hitherto we report the isolation of two new phenyl polyketide lactones, plakinidones B (**2**) and C (**3**), along with the previously described plakinidone (**1**)[5,6] and plakortolide F (**4**),[2b,7] from the $CHCl_3$ extract. Preliminary NMR (^1H and ^{13}C) and EIMS analyses of these lactones immediately revealed their close structural relatedness and in particular the presence in **1**–**4** of a terminal hydroxylated phenyl ring and of a five-membered tetronic acid ring (i.e., 3-methyl-4-hydroxy-2(5*H*)-furanone) in metabolites **1**–**3**.[8]

Received: April 7, 2017

ACS Publications © XXXX American Chemical Society and American Society of Pharmacognosy

DOI: 10.1021/acs.jnatprod.7b00300
J. Nat. Prod. XXXX, XXX, XXX–XXX



Furthermore, a bioactivity-directed fractionation based on anti-TB activity of the aforementioned sponge conglomerate collected in 2011 yielded the known hybrid peptide/polyketide smenothiazole A (**5**). This compound, isolated recently by the Costantino group from the Caribbean sponge *Smenospongia aurea*, was fully characterized by 1D and 2D NMR spectroscopic and HRMS analysis.[9]

RESULTS AND DISCUSSION

Extraction of the 2006 sponge collection with $CHCl_3$–MeOH followed by partitioning of the concentrated extract as described above afforded the $CHCl_3$ extract (8.0 g) used in this investigation. In vitro antituberculosis screening of this extract against *Mycobacterium tuberculosis* (*Mtb*) $H_{37}Rv$ at one concentration (64 μg/mL) exhibited >90% inhibition. Successive reversed- and normal-phase silica gel chromatography afforded 926 mg (yield 0.23%) of plakinidone (**1**)[5,6] and 110 mg (yield 0.028%) of plakortolide F (**4**)[2b,7] as major isolates whose IR, EIMS, $[\alpha]_D$, and ^1H and ^{13}C NMR data were in agreement with published values, whereas plakinidones B (**2**) (60 mg, yield 0.015%) and C (**3**) (16 mg, yield 0.004%) were obtained in lower amounts. Interestingly, plakinidones B and C are only the second and third members of the plakinidone family of compounds to be found since the discovery of **1** in 1991 by Kushlan and Faulkner.[5]

The minor compound, plakinidone B (**2**), was obtained as a yellowish optically active semisolid, $[\alpha]^{20}_D$ −10.6 (*c* 1.0, MeOH). The molecular formula $C_{24}H_{36}O_4$ was determined by HRESIMS, from the peak at *m*/*z* 389.2694 $[M + H]^+$. The structural homology between plakinidone (**1**) and plakinidone B (**2**) was evident from the HRMS data, which showed that their molecular masses corresponded to molecular formulas that differ from each other by a CH_2 group. The IR absorption bands at 3600–3100 and 1724 cm^{-1} suggested the presence in **2** of hydroxy and ester carbonyl groups, respectively. In the NMR spectra, two singlet peaks at δ_H 1.39 and 1.65 (Table 1) together with two peaks at δ_C 23.7 and 5.9 implied the presence of two methyl groups. The only other clearly discernible proton signals were attributable to a *p*-hydroxyphenyl group [δ_H 6.95 (d, *J* = 8.4 Hz) and 6.67 (d, *J* = 8.4 Hz)]. In addition, three resolved sets of methylene protons at δ_H 2.48 (t, *J* = 7.4 Hz), 1.69 (t, *J* = 7.9 Hz), and 1.54 (m) together with nine overlapped pairs of methylene protons at δ_H 1.28–1.20 (br m) suggested that a straight dodecyl chain connected the terminal ring subunits.

The ^{13}C NMR spectrum of **2** (Table 1) showed 24 signals, including those for an ester carbonyl [δ_C 177.8 (C)], eight

Table 1. ^{13}C NMR and ^1H NMR for Plakinidone B (2) and Plakinidone C (3) in CD_3OD

	2			3	
position	δ_C, type[a]	δ_H, mult (*J* in Hz)[b]	position	δ_C, type[a]	δ_H, mult (*J* in Hz)[b]
1, 5	130.2 (2 × CH)	6.95, d (8.4)	1	116.5, CH	6.58, d (2.0)
2, 4	116.0 (2 × CH)	6.67, d (8.4)	2	146.0, C	
3	156.2, C		3	144.0, C	
6	134.9, C		4	116.2, CH	6.64, d (8.0)
7	36.0, CH_2	2.48, t (7.4)	5	120.6, CH	6.46, dd (8.0, 2.0)
8	33.0, CH_2	1.54, m	6	135.7, C	
9	30.6, CH_2	1.28–1.20, br m	7	36.3, CH_2	2.43, t (7.6)
10	30.6, CH_2	1.28–1.20, br m	8	33.2, CH_2	1.51, m
11	30.6, CH_2	1.28–1.20, br m	9	27.6, CH_2	1.25–1.20, br m
12	30.6, CH_2	1.28–1.20, br m	10	37.9, CH_2	1.28, m/1.07, m
13	30.6, CH_2	1.28–1.20, br m	11	33.8, CH_2	1.36, m
14	30.6, CH_2	1.28–1.20, br m	12	37.9, CH_2	1.28, m/1.07, m
15	30.6, CH_2	1.28–1.20, br m	13	27.9, CH_2	1.25–1.20, br m
16	30.2, CH_2	1.28–1.20, br m	14	30.8, CH_2	1.25–1.20, br m
17	24.1, CH_2	1.28–1.20, br m	15	24.1, CH_2	1.25–1.20, br m/1.10, br m
18	37.5, CH_2	1.69, t (7.9)	16	37.4, CH_2	1.72, m
19	85.0, C		17	85.0, C	
20	180.2, C		18	179.8, C	
21	96.0, C		19	96.2, C	
22	177.8, C		20	177.6, C	
23	5.9, CH_3	1.65, s	21	5.9, CH_3	1.64, s
24	23.7, CH_3	1.39, s	22	23.7, CH_3	1.39, s
			23	20.1, CH_3	0.82, d (6.5)

[a]Data (δ) measured at 125 MHz; CH_3, CH_2, CH, and C multiplicities were determined by HSQC and DEPT-135 NMR experiments. [b]Data (δ) measured at 500 MHz. *J* values are omitted if the signals overlapped as multiplets. The overlapped signals were assigned from HSQC and HMBC spectra without designating multiplicity.

DOI: 10.1021/acs.jnatprod.7b00300
J. Nat. Prod. XXXX, XXX, XXX–XXX

olefinic carbons [δ_C 180.2 (C), 156.2 (C), 134.9 (C), 130.2 (2 × CH), 116.0 (2 × CH), and 96.0 (C)], and one esterified carbon [δ_C 85.0 (C)]. These carbon signals were subsequently assigned to a *p*-hydroxylphenyl ring and a tetronic acid moiety through COSY, HSQC, and HMBC experiments (Figure 1).

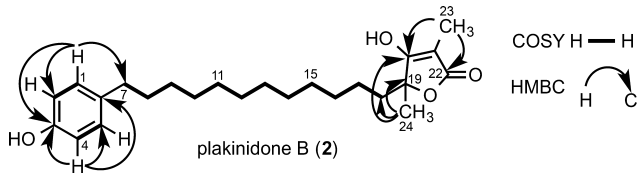
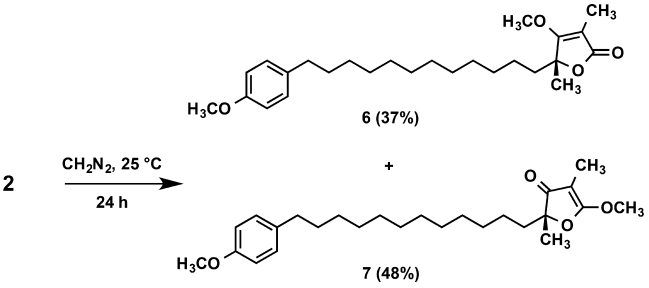


Figure 1. Key COSY (bold) and HMBC (→) correlations for plakinidone B (**2**).

Inspection of the NMR spectra for **2**, when compared to those of compound **1**, revealed minor differences for the alkyl chain, suggesting that **2** has the same five-membered-ring lactone joined to a terminal *p*-hydroxyphenyl ring through a slightly modified hydrocarbon chain. The slight spectral differences observed could be accounted for by the replacement of the branched C_{10} alkyl chain moiety in **1** by an unbranched C_{12} alkyl chain in **2**.

With the planar structure of plakinidone B (**2**) confidently established, we undertook an investigation to establish its absolute configuration unambiguously. Given the high susceptibility of tetronic acid derivatives for air oxidation, we prepared two stable derivatives from a sample of naturally occurring plakinidone B (Scheme 1).[6b] Thus, the natural product was

Scheme 1. Preparation of Dimethylated Derivatives 6 and 7



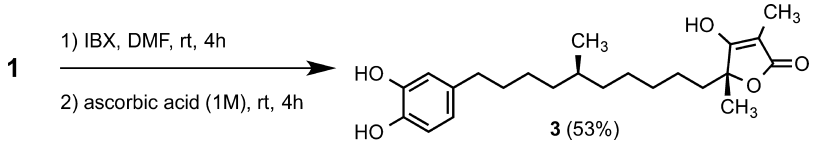
treated with CH_2N_2 to furnish in 85% yield a 1:1.3 mixture of dimethylated adducts **6** and **7**, which were easy to separate using conventional chromatographic methods. The absolute configuration was assigned by vibrational and electronic circular dichroism (VCD and ECD) measurements of dimethylated derivative **6**[10] in combination with density functional theory calculations at the B3LYP/aug-cc-pVDZ/PCM(MeCN) level of theory (Gaussian09 package of programs; see Figures S1−S9 and Tables S1−S5 in the Supporting Information).[11] Our combined efforts and the agreement between the experimental and calculated VCD/ECD spectra of **6** revealed that the absolute configuration of plakinidone B is (19*R*). Furthermore, the nearly identical specific rotation data between compounds **1** and **2** ($[\alpha]^{20}_D$ −13.1 (*c* 0.61, MeOH) versus $[\alpha]^{20}_D$ −10.6 (*c* 1.0, MeOH), respectively) suggest that plakinidone B possesses the same absolute configuration at its only stereogenic center as plakinidone.[6b,12] Therefore, we propose that natural plakinidone B is accurately represented by structure **2**.

The least abundant compound, plakinidone C (**3**), was isolated as a yellowish oil. Its molecular formula was $C_{23}H_{34}O_5$ based on the HREIMS data, which has one oxygen more than that of **1**. The UV spectrum showed similar absorptions to plakinidones at 226 and 256 nm. Comparison of its NMR spectra with those of plakinidone (**1**) indicated that the ^{1}H and ^{13}C NMR data of **3** (Table 1) were strikingly similar except for the substitution pattern of the aromatic ring.[5] The signal for H-2 in plakinidone [δ_H 6.67 (d, *J* = 8.4 Hz)] was no longer present, and δ_C at C-2 (116.0) was deshielded to δ_C 146.0, leading to the assignment of a C-2 hydroxy group. The molecular formula and the key HMBC correlations of H-1 to C-2/C-3/C-5/C-6 and H-4 to C-2/C-3/C-5/C-6 also confirmed the structure of **3**. The configuration of (11*S*,17*R*)-**3**, suggested by $[\alpha]_D$ data comparisons between **1** and **3**, was confirmed from a chemical correlation study wherein the hydroxylation of plakinidone (**1**) with 2-iodoxybenzoic acid (IBX) in dimethylformamide (DMF) led to **3** in 53% yield (Scheme 2) based on TLC, NMR, and $[\alpha]_D$ data.

As previously remarked, our investigation of the compounds responsible for the antitubercular activities of the active extracts led us to the isolation of 3.5 mg (yield 0.005%) of previously known smenothiazole A (**5**).[9] The stereostructural elucidation of compound **5** was determined by de novo analysis of its NMR data and, later, by comparing its NMR data with published data (Table S6). Although **5** has displayed substantial cytotoxicity, the original isolate was not assayed for anti-TB activity. As it happens with many marine natural products, the lack of an adequate supply of material has impeded research into the biological potential of the smenothiazoles.[13] While we attempted to solve the supply issue by total synthesis, all of our efforts to prepare **5** in a practical manner capable of providing an adequate supply of material for biological screening were unsuccessful. Thus, after some consideration, we embarked on a diverted total synthesis of smenothiazole A wherein the chlorine atom in **5** was to be "edited out" chemically through truncation, thus reducing the structural complexity of the natural product. Thus, the aim of this study was to identify a probable pharmacophore for antituberculosis activity in smenothiazole A (**5**) and to deliver improved compounds for biological evaluation.[14]

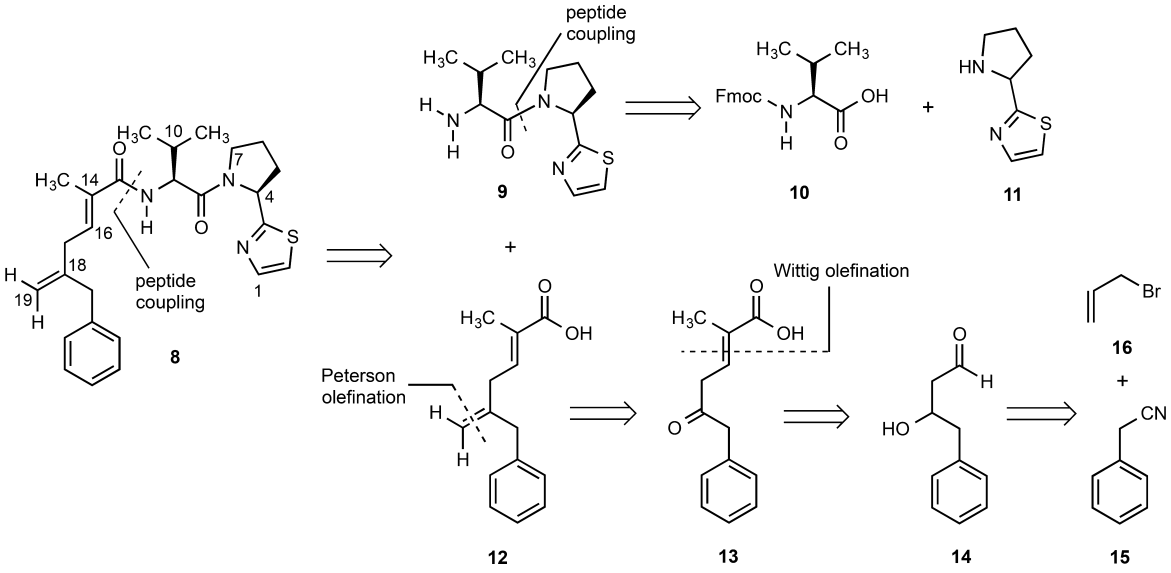
Our retrosynthetic analysis of the total synthesis of dechlorosmenothiazole A (**8**) is delineated in Scheme 3. We envisioned the disconnection of the secondary amide bond to give fragments **9** and **12**. The peptidic fragment **9** could be prepared from commercially available Fmoc-Val-OH (**10**) and 2-pyrrolidin-2-ylthiazole (**11**). The pivotal polyketide segment **12** was envisioned from key C-18/C-19 and C-14/C-16

Scheme 2. Semisynthesis of Plakinidone C (3) from Plakinidone (1)

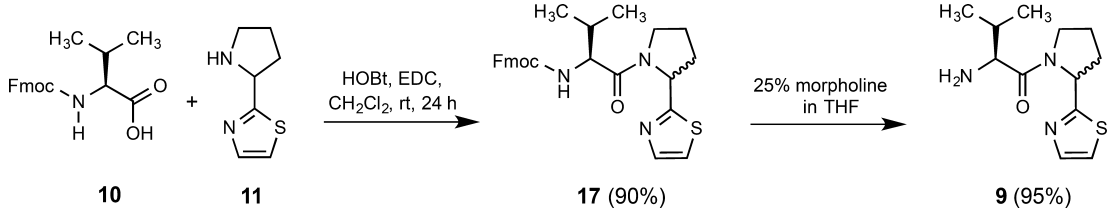


DOI: 10.1021/acs.jnatprod.7b00300
J. Nat. Prod. XXXX, XXX, XXX−XXX

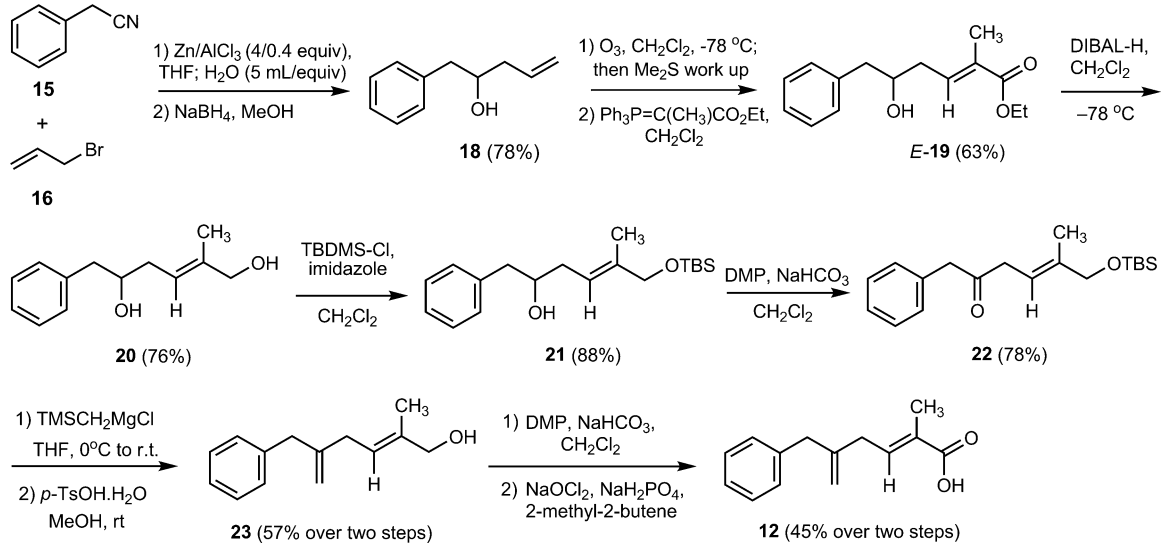
Scheme 3. Retrosynthetic Analysis of Dechloro-smenothiazole A (8)



Scheme 4. Synthesis of Primary Amines 9



Scheme 5. Synthesis of Key Polyketide Segment 12



double-bond disconnections. The precursor to intermediate ketone **13** will be β-hydroxy aldehyde **14**, which in turn could be generated from commercially available benzyl cyanide (**15**) and allyl bromide (**16**).

As outlined in Scheme 3, our synthesis commenced from coupling of commercially available Fmoc-Val-OH (**10**) and 2-pyrrolidin-2-ylthiazole (**11**) in the presence of peptide coupling reagents (ethylene dichloride (EDC) and hydroxybenzotriazole (HOBt))[15] to afford protected intermediate **17** in 90% yield. Removal of the Fmoc protecting group with 25% morpholine in tetrahydrofuran (THF) furnished primary amines **9** in 95% yield as a 1:1 mixture of epimers (Scheme 4).

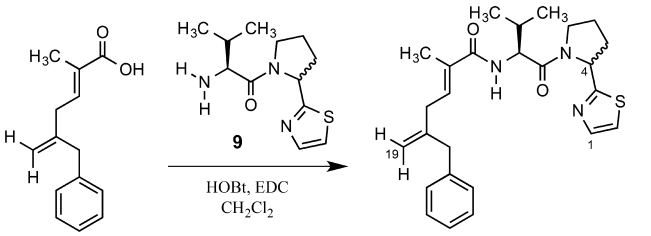
With the epimeric mixture of amines **9** in hand, we took aim at the synthesis of key polyketide segment **12**. As shown in Scheme 5, benzyl cyanide (**15**) was coupled with allyl bromide (**16**) through a Barbier-type reaction aided by $Zn/AlCl_3$ to afford the desired β,γ-unsaturated ketone (not shown) in 93% yield.[16] The known ketone[16] was treated with $NaBH_4$ in MeOH to give racemic homoallylic alcohol **18**, also a known compound, in 78% yield.[17] The one-pot ozonolysis–Wittig

DOI: 10.1021/acs.jnatprod.7b00300
J. Nat. Prod. XXXX, XXX, XXX−XXX

olefination[18,19] of **18** yielded the desired α,β-unsaturated ester **19** (through intermediate **14**) as a mixture of *E*- and *Z*-isomers (*E*:*Z*, 18:1). Fortunately, the two isomers could be separated by flash chromatography over silica gel easily, and pure *E*-**19** was obtained in 63% yield. Compound *E*-**19** was then immediately reduced with diisobutylaluminum hydride (DIBAL-H) in CH_2Cl_2 to furnish alcohol **20** in 76% yield, which was then protected regioselectively with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) in the presence of imidazole to give **21** in 88% yield. Alcohol **21** was later oxidized to ketone **22** with Dess-Martin periodinane in 78% yield.[20] Treatment of ketone **22** using the Peterson olefination methodology[21] followed by exposure of the intermediate tertiary alcohol (not shown) to catalytic amounts of $TsOH{\cdot}H_2O$ in MeOH afforded the desired olefin **23** in 57% yield over two steps. To conclude, a two-step oxidation sequence of allylic alcohol **23** with Dess-Martin periodinane in CH_2Cl_2 followed by exposure to sodium chlorite furnished the desired polyketide portion **12** in 45% yield.[22]

Finally, following a standard protocol, key segments **9** and **12** were coupled to afford **8** in 78% yield as a 1:1 mixture of epimers at C-4 (Scheme 6). A side-by-side comparison of our ^{13}C NMR spectroscopic data for **8** (mixture of epimers) with those for natural smenothiazole A (**5**) revealed excellent agreement (Figure S10).[23]

Scheme 6. Synthesis of Dechloro-smenothiazole A (8) from the Coupling of Segments 9 and 12



In Vitro Inhibition of *Mycobacterium tuberculosis* H_{37}RV. Pure isolated natural products **1**–**5** and the synthetic mixture of epimers **8** were analyzed using a serial dilution from 128 to 1 μg/mL against *Mtb* H_{37}RV in order to compare the effects of each of the compounds and to select the most promising active substance, using rifampicin (RMP) as a positive control. The percentage inhibition at each concentration was used to determine the MIC (the lowest compound concentration that prevents visible growth of the bacterium) of each compound in a broth microdilution Alamar blue assay (MABA).[24] As shown in Table 2, neither compounds **1**–**4** nor **8** showed significant effects on *Mtb* H_{37}Rv growth.[25] On the other hand, smenothiazole A (**5**) gave the best (lowest) MIC value (4.1 μg/mL). Compound **5** was then assessed for potential cytotoxicity to human cells through the use of cultured Vero cells, giving an IC_{50} value of >128 μg/mL. Given its good MIC and low cytotoxicity, **5** is a potential candidate for efficacy studies in mice.

CONCLUSION

A significant finding of this study was the discovery of the second and third examples of a rare class of natural products, namely, the plakinidones.[5,6] Screening of the antituberculosis activity of smenothiazole A (**5**) identified this interesting hybrid peptide/polyketide as a new lead compound endowed with high activity toward *Mtb* H_{37}Rv, exhibiting an MIC value of 4.1 μg/mL. Interestingly, the lack of in vitro activity for synthetic derivative **8** suggests that the presence of an electron-withdrawing group, such as a chlorine atom, at the C-19 position of smenothiazole A (**5**) is essential for anti-TB activity. These preliminary results validate our contention that the vinyl chloride functionality of **5** could represent a new pharmacophore for antituberculosis activity and thus should be taken into consideration by research groups that are exploring the utility of future peptide/polyketide-based compounds as effective anti-TB drugs.[26,27]

Table 2. In Vitro Antituberculosis Activity for Pure Isolated Compounds 1–5 and Synthetic Compound 8[a]

compound	MABA MIC (μg/mL)[b]
plakinidone (**1**)	>128
plakinidone B (**2**)	>128
plakinidone C (**3**)	>128
plakortolide F (**4**)	59.0[c]
smenothiazole A (**5**)	4.1
dechloro-smenothiazole A (**8**)[d]	>128
Ctrl+[e]	0.1

[a]*Mtb* H_{37}Rv strain. [b]Values are means of three experiments. [c]Value taken from ref 2b. [d]Mixture of two epimers. [e]Rifampicin (RMP) was used as the positive control.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Analytical Autopol IV. The UV spectra were recorded on a Shimadzu UV-2401 PC UV–visible spectrometer. The ECD spectra in MeCN were measured in a quartz cell with a path length of 0.1 cm between 300 and 180 nm at room temperature (rt) on a JASCO J-815 circular dichroism spectrometer. The VCD spectra were measured in CD_3CN on a ChiralIR-2X FT-VCD spectrometer (BioTools Inc.). The IR spectra were measured on a Bruker FT-IR spectrometer. The NMR spectra were recorded on a Bruker DRX-500 FT-NMR or a Bruker Ascend 700 FT-NMR spectrometer. $CDCl_3$ was used both as a solvent and as an internal reference at δ_H 7.26 and δ_C 77.0. The HRESIMS data were obtained at the Mass Spectrometry Laboratory of the School of Chemical Sciences, University of Illinois, using a VG 70-VSE (EI+, 70 eV). TLC was carried out on precoated silica gel 60 GF_{254} or RP-18 silica gel 60 GF_{254} (Analtech). Gravity column chromatography was performed with normal- and reversed-phase (RP-18) silica gel (35–75 mesh, Analtech). The plates were analyzed under UV light, treated with I_2 vapors, or sprayed with phosphomolybdic acid solution in EtOH. HPLC was carried out on an Agilent 1260 Infinity equipped with an Agilent 1260 photodiode array detector using HPLC-grade solvents. All chemical reagents were purchased from Sigma-Aldrich Co.

Sponge Material (First Collection), Extraction, and Isolation. Fresh specimens of the sponge *Plakortis symbiotica* (Vicente, Zea & Hill, 2016) (phylum Porifera; class Homoscleromorpha; order Homosclerophorida; family Plakinidae) in association with *Xestospongia deweerdtae* (Lehnert & van Soest, 1999) (phylum Porifera; class Demospongiae; subclass Heteroscleromorpha; order Haplosclerida; family Petrosiidae) were collected by hand using scuba at depths of 90–100 ft off Mona Island, Puerto Rico, in July 2006.[4] A voucher specimen (No. IM06-09) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. In prior reports with these symbiotic sponges we originally classified the species *P. symbiotica* as *P. halichondrioides*.[2] The specimens were frozen and lyophilized prior to extraction. The dry specimens (395 g) were cut into small pieces and blended in a mixture of $CHCl_3$–MeOH (1:1) (11 × 1 L). After filtration, the crude extract was concentrated and

DOI: 10.1021/acs.jnatprod.7b00300
J. Nat. Prod. XXXX, XXX, XXX–XXX

stored under vacuum to yield a dark gum (100 g), which was suspended in H_2O (2 L) and extracted with *n*-hexane (3 × 2 L) and $CHCl_3$ (3 × 2 L). Concentration of the $CHCl_3$ extract under reduced pressure yielded 8.0 g of a dark brown oil, which was chromatographed over RP-18 silica gel (100 g) using mixtures of MeOH–H_2O of increasing polarity (70–100%). A total of 10 fractions (I–X) were generated on the basis of TLC and ^{1}H NMR analyses. Initial purification of fraction IV(D) (228 mg) by column chromatography (silica gel, 5.0 g) using a gradient mixture of $CHCl_3$–MeOH (90:10 → 80:20) afforded five subfractions, denoted as D(1)–D(5). Purification of subfraction D(1) (30 mg) through a short plug of silica gel (1.0 g) eluting with $CHCl_3$–MeOH (95:5) afforded plakinidone C (**3**) (16 mg, yield 0.004%). Careful scrutiny of combined spectroscopic data revealed that fractions V(E) and VI(F) consisted of known plakinidone (**1**) (926 mg, yield 0.23%).[5,6] Purification of fraction VII (700 mg) by silica gel (16.0 g) column chromatography using a gradient mixture of $CHCl_3$–MeOH (100:0 → 90:10) afforded known cyclic peroxide plakortolide F (**4**) (110 mg, yield 0.028%).[2b,7] Further purification of fraction VIII(H) (760 mg) by RP-18 silica gel (20 g) column chromatography in MeOH–H_2O (9:1) afforded five subfractions, denoted as H(1)–H(5). Subfraction H(3) (100 mg) was purified by RP-18 silica gel (4.0 g) column chromatography in MeOH–H_2O (8:2) to afford plakinidone B (**2**) (60 mg, yield 0.015%). Interestingly, no traces of known smenothiazole A (**5**)[9] were detected in any of the extracts obtained using animal material stemming from this collection.

*Plakinidone (**1**):* $[\alpha]^{20}_D$ −12 (*c* 0.6, MeOH); lit.[6b] $[\alpha]^{20}_D$ −13.1 (*c* 0.6, MeOH).

*Plakinidone B (**2**):* yellowish semisolid; $[\alpha]^{20}_D$ −10.6 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.74), 256 (4.48) nm; IR (film) ν_{max} 3600–3100 (br), 2926, 2854, 1878 (weak), 1724, 1661, 1515, 1456, 1374, 1228, 1105, 828, 765 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Table 1; LREIMS *m*/*z* 388 (3), 372 (4), 318 (28), 304 (18), 260 (6), 246 (6), 149 (3), 133 (7), 120 (12), 107 (100); HRESIMS *m*/*z* 389.2694 $[M + H]^+$ (calcd for $C_{24}H_{37}O_4$, 389.2692).

*Plakinidone C (**3**):* yellowish oil; $[\alpha]^{20}_D$ −4.7 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 201 (4.18), 226 (3.99), 256 (3.61) nm; IR (film) ν_{max} 3290 (br), 2928, 2856, 1720, 1660, 1519, 1454, 1284, 1112, 949, 758 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Table 1; LREIMS *m*/*z* 390 (86), 306, 123 (100); HREIMS *m*/*z* 390.2400 $[M]^+$ (calcd for $C_{23}H_{34}O_5$, 390.2407).

*Plakortolide F (**4**):* $[\alpha]^{20}_D$ +5.2 (*c* 0.6, $CHCl_3$); lit.[2b] $[\alpha]^{20}_D$ +5.7 (*c* 1.1, $CHCl_3$).

Sponge Material (Second Collection), Extraction, and Isolation. Fresh specimens of the sponge consortium *P. symbiotica*–*X. deweerdtae* were collected by hand using scuba at depths of 90 ft off Mona Island, Puerto Rico, in June 2011. A voucher specimen (No. IM11-04) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. Freeze-dried specimens (1.59 kg) were cut into small pieces and blended in a mixture of $CHCl_3$–MeOH (1:1) (36 L). After filtration, the extract was concentrated and stored under vacuum to yield a dark gum (323 g), which was suspended in H_2O and extracted with *n*-hexane (16 L). Concentration under reduced pressure yielded the *n*-hexane extract (69.0 g) as a dark brown oil, a portion of which (16.3 g) was chromatographed over silica gel (250 g) using mixtures of *n*-hexane–acetone of increasing polarity (0–100%). A total of 12 fractions (I–XII) were generated on the basis of TLC and ^{1}H NMR analysis. Further purification of fraction VI (1.3 g) by silica gel (30.0 g) column chromatography in $CHCl_3$–EtOAc (100:0; 95:5; 90:10) afforded six subfractions, denoted as A–F. Fraction F (700 mg) was purified by column chromatography over silica gel (10.0 g), eluting with an isocratic mixture of $CHCl_3$–EtOAc (9:1), to give four subfractions, denoted as F1–F4. Subfraction F4 (287 mg) was purified sequentially by column chromatography over cyano silica gel (4.0 g) using isocratic *n*-hexanes–isopropyl alcohol (96:4) as mobile phase followed by purification of subfraction F4b (25 mg) by chiral-phase HPLC (stationary phase, (R,R)-Whelk-O 1 10 μm, 150 × 4.6 mm i.d. (Regis Technologies, Inc.); mobile phase, MeOH–H_2O (85:15); flow rate, 1 mL/min; detection, UV 254 nm) to afford pure smenothiazole A (**5**) (t_R = 22.4 min, 3.5 mg, yield 0.005%).[9]

A cursory NMR- and chromatography-based dereplication analysis of a small portion (100 mg) of the gross $CHCl_3$ extract (13.1 g) led us to select four compounds as being potentially of interest for isolation regarding their putative novelty. However, after gathering non-quantified amounts suitable for full structure elucidation and identification, the compounds were quickly found to be previously extracted plakinidone (**1**), plakinidone B (**2**), plakinidone C (**3**), and plakortolide F (**4**).

*Smenothiazole A (**5**):* colorless oil; $[\alpha]^{20}_D$ −45 (*c* 0.8, $CHCl_3$) (specific rotation data were not provided for the original isolate);[9] UV (MeOH) λ_{max} 206 nm (log ε 4.35); IR (film) ν_{max} 3330, 2963, 2929, 1724, 1628, 1497, 1431, 1321, 1178, 1077, 734, 702 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Table S6; LREIMS *m*/*z* 485 (26), 304 (43), 233 (66), 182 (45), 154 (27), 91 (100); HREISMS *m*/*z* 486.1987 $[M + H]^+$ (calcd for $C_{26}H_{33}ClN_3O_2S$, 486.1982).

Methylation of Plakinidone B (2). To a solution of plakinidone B (35 mg, 0.090 mmol) in $CHCl_3$ (5 mL) was added a solution of excess diazomethane in ether (25 mL), and the resulting mixture was stirred at 25 °C for 24 h. The reaction mixture was concentrated in vacuo, and the oily residue left over was chromatographed by silica gel column chromatography (1.0 g) eluting with mixtures of $CHCl_3$–*n*-hexanes of increasing polarity (8:2; 9:1; 10:0) to afford pure compounds **6** (14 mg, 37% yield) and **7** (18.2 mg, 48% yield) as colorless oils; minor isomer **6** $[\alpha]^{20}_D$ −12 (*c* 0.9, $CHCl_3$); UV (1.7 × 10^{-4} M, MeCN) λ_{max} (ε) 195 (41 200), 226 (16 700), 278 (1650), 285 (1400) nm; ECD (1.7 × 10^{-4} M, MeCN), λ_{max} ($\Delta\varepsilon$) 191 (−1.78), 199 (−1.1), 226 (+0.31) nm; IR (film) ν_{max} 2919, 2849, 1742, 1665, 1511, 1465, 1388, 1315, 1246, 1036, 979, 814 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.09 (2H, d, *J* = 8.4 Hz, H-1), 6.82 (2H, d, *J* = 8.4 Hz, H-2), 4.10 (3H, s, OCH_3, H_3-25), 3.78 (3H, s, OCH_3, H_3-26), 2.53 (2H, t, *J* = 7.6 Hz, H_2-7), 1.72/1.60 (2H, m, H_2-18), 2.00 (3H, s, H_3-23), 1.57 (2H, m, H_2-8), 1.38 (3H, s, H_3-24), 1.29–1.23 (18H, br envelope, H_2-9 through H_2-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.0 (C, C-20), 174.3 (C, C-22), 157.5 (C, C-3), 135.0 (C, C-6), 129.2 (2 × CH, C-1), 113.6 (2 × CH, C-2), 96.0 (C, C-21), 83.1 (C, C-19), 58.9 (OCH_3, C-25), 55.2 (OCH_3, C-26), 36.8 (CH_2, C-18), 35.0 (CH_2, C-7), 31.7 (CH_2, C-8), 29.6–29.3 (8 × CH_2, C-9 through C-16), 23.5 (CH_3, C-24), 23.0 (CH_2, C-17), 8.5 (CH_3, C-23); LREIMS *m*/*z* 416 (6), 177 (26), 149 (100), 130 (22), 121 (32), 98 (26); major isomer **7** $[\alpha]^{20}_D$ +22 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 201 (4.00), 224 (3.87), 264 (4.08) nm; IR (film) ν_{max} 2924, 2853, 1701, 1610, 1512, 1476, 1394, 1367, 1246, 1197, 1037, 978, 819 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.09 (2H, d, *J* = 8.4 Hz, H-1), 6.82 (2H, d, *J* = 8.4 Hz, H-2), 4.00 (3H, s, OCH_3, H_3-25), 3.78 (3H, s, OCH_3, H_3-26), 2.53 (2H, t, *J* = 7.6 Hz, H_2-7), 1.73 (2H, m, H_2-18), 1.58 (3H, s, H_3-23), 1.56 (2H, m, H_2-8), 1.39 (3H, s, H_3-24), 1.29–1.23 (18H, br envelope, H_2-9 through H_2-17); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 201.0 (C, C-20), 179.3 (C, C-22), 157.5 (C, C-3), 135.0 (C, C-6), 129.2 (2 × CH, C-1), 113.6 (2 × CH, C-2), 93.0 (C, C-21), 87.4 (C, C-19), 55.6 (OCH_3, C-25), 55.2 (OCH_3, C-26), 36.3 (CH_2, C-18), 35.0 (CH_2, C-7), 31.7 (CH_2, C-8), 29.6–29.2 (8 × CH_2, C-9 through C-16), 23.0 (CH_2, C-17), 22.0 (CH_3, C-24), 3.9 (CH_3, C-23); LREIMS *m*/*z* 416 (30), 177 (11), 155 (21), 149 (36), 142 (59), 135 (14), 121 (100).

Semisynthesis of Plakinidone C (3). IBX (15 mg, 0.030 mmol) was added in the dark to a solution of plakinidone (**1**) (10 mg, 0.027 mmol) in DMF (3 mL). After stirring the reaction mixture for 4 h at 25 °C the red-colored solution was treated with L-ascorbic acid (0.25 mL, 1 M in H_2O) and left stirring until the color turned light yellow (~4 h). After extracting with EtOAc (3 × 15 mL) the organic layer was concentrated to give an oil, which was passed through a short plug of silica gel (0.7 g) using a mixture of $CHCl_3$–MeOH (4.9:0.1) to yield plakinidone C (**3**) (5.5 mg, 53% yield). Upon careful comparison, the overall spectroscopic (IR, UV, EIMS, ^{1}H and ^{13}C NMR), TLC, and specific rotation data ($[\alpha]^{20}_D$ −4.8 (*c* 0.8, MeOH)) of synthetic **3** and the natural product were in excellent agreement.

Compound 17. To a solution of 2-pyrrolidin-2-ylthiazole (**11**, 325 mg, 2.11 mmol, 1 equiv) in dry CH_2Cl_2 (25 mL) were added Fmoc-

DOI: 10.1021/acs.jnatprod.7b00300
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Val-OH (**10**, 832 mg, 1 equiv), EDC (406 mg, 1 equiv), and HOBt (285 mg, 1 equiv), and the mixture was stirred at rt for 48 h. The reaction mixture was then concentrated to dryness, basified with saturated aqueous $NaHCO_3$ (50 mL), and extracted with EtOAc (3 × 50 mL). The crude product obtained after solvent removal was purified by silica gel (5 g) column chromatography using $CHCl_3$ to afford **17** (900 mg, 90%) as a white semisolid, which was used in the following step without further purification.

Compound 9. To a solution of **17** (890 mg, 1.87 mmol) in THF (5 mL) was added 25% morpholine in THF (10 mL). After stirring for 8 h at 25 °C the mixture was concentrated under reduced pressure to give a crude material that was purified by silica gel (5 g) column chromatography with 95:5 $CHCl_3$–MeOH to afford compound **9** (449 mg, 95%) as a 1:1 mixture of epimers as a colorless foam: IR (film) ν_{max} 3373, 3302, 3081, 2960, 2874, 1643, 1499, 1430, 1364, 1265, 1203, 1134, 1055, 890, 830, 729 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.47 (1H, d, J = 3.3 Hz)/7.46 (1H, d, J = 3.3 Hz); 7.03 (1H, d, J = 3.3 Hz)/7.02 (1H, d, J = 3.3 Hz); 5.35 (1H, dd, J = 7.9, 2.4 Hz)/5.29 (1H, dd, J = 7.5, 2.0 Hz); 3.39–3.54 (4H, m); 3.21 (1H, d, J = 4.9 Hz)/3.12 (1H, d, J = 6.3 Hz); 2.17 (1H, m)/2.02 (1H, m); 1.98–1.86 (4H, m); 1.77–1.69 (4H, m); 0.82 (3H, d, J = 6.8 Hz)/0.79 (3H, d, J = 6.8 Hz); 0.74 (3H, d, J = 6.8 Hz)/0.74 (3H, d, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.9/173.7, 172.0/171.5, 141.7/141.6, 118.4/118.2, 58.2/58.0, 57.7/57.6, 46.5/46.3, 31.3/31.2, 31.1/30.9, 24.0/23.7, 19.7/19.3, 16.8/16.0; LRESIMS m/z 254.6 $[M + H]^+$; HREISMS m/z 254.1329 $[M + H]^+$ (calcd for $C_{12}H_{20}N_3OS$, 254.1327).

1-Phenylpent-4-en-2-ol (18). To a suspension of zinc powder (1.2 g, 18.8 mmol, 4 equiv) in dry THF (6 mL) kept at 0 °C under a N_2 atmosphere was added allyl bromide (**16**, 555 mg, 4.69 mmol, 1 equiv). After stirring for 3 h at 0–25 °C, benzyl cyanide (**15**, 535 mg, 4.69 mmol, 1 equiv) in dry THF (1 mL) was added dropwise. After stirring for 1.5 h the mixture was cooled to 0 °C, and then $AlCl_3$ (248 mg, 1.88 mmol, 0.4 equiv) was slowly added (*exothermic!*). The mixture was stirred for the next 1.5 h while allowing the temperature to rise to rt. After adding H_2O (15 mL), the reaction mixture was stirred for another 1.5 h, more H_2O was added (60 mL), and the mixture was extracted with EtOAc (3 × 75 mL). The combined organic layer was dried ($MgSO_4$), and the solvent removed under reduced pressure. The yellow oil obtained was identified as known 1-phenylpent-4-en-2-one (700 mg, 93%),[16] which was used in the following step without further purification. To a solution of the latter compound (700 mg, 4.37 mmol, 1 equiv) in MeOH (10 mL) at 0 °C was added $NaBH_4$ (200 mg, 5.25 mmol, 1.2 equiv). The reaction mixture was stirred at 0 °C for 3 h and then concentrated under reduced pressure, diluted with H_2O (25 mL), and extracted with $CHCl_3$ (25 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The crude was purified by flash silica gel column chromatography (5 g, 95:5 *n*-Hex–EtOAc) to give homoallylic alcohol **18** (554 mg, 78%) as a colorless oil. The ^1H and ^{13}C NMR spectroscopic properties were in agreement with data published in the literature.[17]

Compound 19. Through a stirred solution of homoallylic alcohol **18** (550 mg, 3.39 mmol) and $NaHCO_3$ (spatula tipful) in CH_2Cl_2 (15 mL) kept at −78 °C was bubbled a stream of ozone. Ozone treatment was terminated when the reaction mixture maintained a blue color for 1 min. Thereafter, it was replaced by N_2, and the solution stirred until the color dissipated (~2 h). To the clear mixture was added dimethylsulfide (2.5 mL), and the stirring continued for 36 h while the temperature ranged from −78 °C to rt. The crude β-hydroxy aldehyde **14** obtained after solvent removal was stored under vacuum for over 12 h (610 mg, 86%), which was then used in the next reaction without further purification. To the latter intermediate (600 mg, 2.86 mmol, 1 equiv) in dry CH_2Cl_2 (15 mL) was added phosphorus ylide (carbethoxyethylidene)triphenylphosphorane (1.45 g, 4.0 mmol, 1.4 equiv). After the resulting solution was stirred at 25 °C for 48 h, silica gel (~0.3 g) was added and the solvent rotoevaporated. The crude obtained was chromatographed over silica gel (10 g) and eluted with mixtures of *n*-Hex–EtOAc of increasing polarity (95:5, 90:10, and 85:15) to afford esters *Z*-**19** (25 mg, 3.5%) and *E*-**19** (450 mg, 63%) as clear, colorless oils: minor isomer *Z*-**19** IR (film) ν_{max} 3444 (br), 3086, 3062, 3028, 2981, 2928, 1710, 1647, 1603, 1496, 1454, 1373, 1228, 1132, 1081, 907, 859, 746, 701 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 7.30–7.22 (5H, m), 6.06 (1H, dt, J = 7.8, 1.4 Hz), 4.19 (2H, q, J = 7.1 Hz), 3.94 (1H, quintet, J = 6.7 Hz), 2.80 (1H, d, J = 6.5 Hz), 2.63 (1H, q br, J = 7.8 Hz), 2.49 (1H, br s, exchangeable), 1.94 (3H, s), 1.29 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 168.4, 138.4, 138.3, 129.9, 129.4, 128.5, 126.4, 72.3, 60.4, 44.0, 36.4, 20.6, 14.2; LRESIMS m/z 271.6 $[M + Na]^+$, 249.6 $[M + H]^+$, 231.6 $[M + H - H_2O]^+$; major isomer *E*-**19** IR (film) ν_{max} 3454 (br), 3062, 3028, 2982, 2933, 1707, 1649, 1603, 1496, 1454, 1368, 1276, 1125, 1038, 918, 867, 740, 701 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.30–7.21 (5H, m), 6.86 (1H, dt, J = 7.4, 1.4 Hz), 4.18 (2H, q, J = 7.1 Hz), 3.97 (1H, quintet), 2.83 (1H, dd, J = 13.4, 4.9 Hz), 2.73 (1H, dd, J = 13.4, 8.1 Hz), 2.39 (2H, t, J = 7.1 Hz), 2.07 (1H, br s, exchangeable), 1.84 (3H, s), 1.29 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.9, 137.7, 137.9, 129.8, 129.5, 128.5, 126.5, 71.7, 60.5, 43.5, 35.8, 14.2, 12.6; LRESIMS m/z 271.6 $[M + Na]^+$, 249.6 $[M + H]^+$, 231.6 $[M + H - H_2O]^+$.

Compound 20. A solution of ethyl ester *E*-**19** (600 mg, 2.42 mmol, 1 equiv) in dry CH_2Cl_2 (5.0 mL) was cooled to −78 °C and treated with 1.0 M DIBAL-H in THF (7.0 mL, 3 equiv) dropwise over a period of 5 min. The resulting solution was stirred at −78 °C for another 20 min, slowly warmed to rt, and then stirred for an additional 20 h. The reaction was then carefully quenched in sequence with MeOH (0.5 mL), 10% NaOH (15 mL), and H_2O (35 mL). After extraction with EtOAc (3 × 40 mL) the combined organic layer was dried ($MgSO_4$) and concentrated to leave an oily residue, which was purified by column chromatography over silica gel (4.5 g, *n*-hexanes–EtOAc, 7:3; 6:4; 1:1) to afford pure diol **20** (380 mg, 76%) as a colorless oil: IR (film) ν_{max} 3446 (br), 3062, 3028, 2918, 2861, 1603, 1496, 1453, 1356, 1230, 1046, 1006, 896, 863, 744, 701 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 7.29–7.20 (5H, m), 5.47 (1H, t, J = 7.7 Hz), 3.94 (2H, s), 3.85 (1H, m), 2.75 (2H, m), 2.22 (2H, m), 1.63 (3H, s); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 138.5, 137.3, 129.3, 128.3, 126.2, 121.3, 72.4, 68.1, 43.3, 34.6, 13.9; LRESIMS m/z 171.1 $[M + H - 2H_2O]^+$, 229.1 $[M + Na]^+$, 245.1 $[M + K]^+$.

Compound 21. To a solution of diol **20** (590 mg, 2.86 mmol, 1 equiv), imidazole (253 mg, 1.3 equiv), and 4-dimethylaminopyridine (DMAP) (35 mg, 0.1 equiv) in CH_2Cl_2 (6 mL) kept at 0 °C was added 1.0 M *tert*-butyldimethylsilyl chloride in THF (3.72 mL, 1.3 equiv) over 5 min. The mixture was stirred for 5 h at 0 °C and later quenched with saturated NH_4Cl (30 mL). After the aqueous layer was extracted with EtOAc (3 × 30 mL) the combined organic layer was washed with H_2O (30 mL) and brine (30 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by column chromatography over silica gel (3.5 g, *n*-hexanes–EtOAc, 1:0; 95:5; 90:10) to yield silyl ether **21** (810 mg, 88%) as a colorless oil: IR (film) ν_{max} 3442 (br), 3063, 3028, 2929, 2857, 1603, 1496, 1471, 1362, 1254, 1071, 1006, 838, 776, 743, 700, 668 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 7.33–7.25 (5H, m), 5.53 (1H, t, J = 7.4 Hz), 4.07 (2H, s), 3.88 (1H, m), 2.85 (1H, dd, J = 13.6, 4.7 Hz), 2.72 (1H, dd, J = 13.6, 8.0 Hz), 2.29 (2H, m), 1.65 (3H, s), 0.94 (9H, s), 0.10 (6H, s); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 138.6, 137.6, 129.4, 128.4, 126.3, 119.6, 72.5, 68.3, 43.2, 34.9, 25.9, 18.4, 13.7, −5.3; LRESIMS m/z 359.2 $[M + K]^+$, 343.2 $[M + Na]^+$.

Compound 22. To a stirred solution of **21** (765.3 mg, 2.39 mmol, 1 equiv) in CH_2Cl_2 (10 mL) kept at 0 °C were added Dess-Martin periodinane (1.12 g, 2.63 mmol, 1.1 equiv) and $NaHCO_3$ (402 mg, 4.78 mmol, 2 equiv). The mixture was stirred for 2 h, diluted with saturated $Na_2S_2O_3$ (25 mL), and extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with saturated $NaHCO_3$ (25 mL) and brine (25 mL), dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by column chromatography over silica gel (3 g, *n*-hexane–EtOAc, 1:0; 98:2; 96:4) to give ketone **22** (589 mg, 78%) as a colorless oil: IR (film) ν_{max} 2954, 2930, 2857, 1717, 1700, 1685, 1456, 1254, 1098, 838, 779, 743, 702 cm^{-1}; ^1H NMR ($CDCl_3$, 700 MHz) δ 7.35–7.23 (5H, m), 5.64 (1H, t, J = 7.2 Hz), 4.07 (2H, s), 3.74 (2H, s), 3.22 (2H, d, J = 7.2 Hz), 1.58 (3H, s), 0.94 (9H, s),

DOI: 10.1021/acs.jnatprod.7b00300
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0.10 (6H, s); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 206.4, 138.6, 134.2, 129.4, 128.7, 127.0, 115.4, 68.6, 49.5, 41.3, 25.9, 18.4, 13.7, −5.3; LRESIMS m/z 357.2 $[M + K]^+$, 341.2 $[M + Na]^+$; HREISMS m/z 341.1915 $[M + Na]^+$ (calcd for $C_{19}H_{30}O_2SiNa$, 341.1913).

Compound 23. A cooled (0 °C) solution of ketone **22** (338.9 mg, 1.07 mmol, 1 equiv) in THF (3 mL) kept under a N_2 atmosphere was treated with 1.0 M $TMSCH_2$−MgCl in Et_2O (7.46 mL, 7 equiv) and stirred at 0 °C for 1 h. Thereafter, the solution was stirred for 5 h while allowing the temperature to rise to rt. After quenching with H_2O (5 mL) the mixture was diluted with EtOAc (30 mL), and the organic layer was washed with saturated NH_4Cl (3 × 20 mL), dried ($MgSO_4$), and concentrated to afford the intermediate tertiary alcohol (structure not shown), which was used in the next step without further purification. A mixture of the latter intermediate (346 mg, 0.852 mmol) and *p*-TsOH·H_2O (8.5 mg, 0.0426 mmol) in MeOH (2 mL) was stirred for 30 min at 25 °C. The reaction mixture was diluted with saturated $NaHCO_3$ (25 mL) and extracted with EtOAc (2 × 25 mL). The combined organic layer was washed with brine (15 mL), dried over $MgSO_4$, and concentrated, and the residue left over was passed through a short plug of silica gel (1.0 g, *n*-hexanes−EtOAc, 1:0 → 8:2) to furnish pure **23** (97.5 mg, 57% over two steps) as a colorless oil: IR (film) ν_{max} 3447 (br), 3064, 3028, 2916, 2859, 1696, 1645, 1602, 1495, 1453, 1434, 1248, 1064, 1017, 1002, 894, 862, 838, 740, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.28−7.21 (5H, m), 5.43 (1H, t, J = 7.4 Hz), 4.85 (1H, s), 4.78 (1H, s), 4.00 (2H, d, J = 5.6 Hz), 3.35 (2H, s), 2.71 (2H, d, J = 7.4 Hz), 1.58 (3H, s); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 147.6, 139.6, 136.3, 129.0, 128.3, 126.1, 123.3, 111.9, 68.8, 43.1, 33.8, 13.6; LRESIMS m/z 241.3 $[M + K]^+$, 225.5 $[M + Na]^+$, 185.5 $[M + H - H_2O]^+$.

Compound 12. A 25 °C mixture of allyl alcohol **23** (57.5 mg, 0.284 mmol, 1 equiv), Dess-Martin periodinane (157 mg, 1.3 equiv), and $NaHCO_3$ (48 mg, 2 equiv) in dry CH_2Cl_2 (2 mL) was stirred for 1 h. The reaction mixture was then diluted with saturated $Na_2S_2O_3$ (20 mL) and extracted with EtOAc (3 × 20 mL), and the combined extracts were washed with saturated $NaHCO_3$ (20 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated to dryness. The crude product obtained was purified by silica gel (1.0 g) using *n*-hexanes−EtOAc (9:1) to afford the desired aldehyde intermediate (51.4 mg), which was used without delay in the next step. A solution of the latter aldehyde (51.4 mg, 0.257 mmol, 1 equiv) in *t*-BuOH (0.5 mL) was treated sequentially with 2 M 2-methyl-2-butene in THF (3.0 mL, 6.168 mmol), $NaClO_2$ (70 mg, 3 equiv), and NaH_2PO_4 (123.3 mg, 4 equiv) dissolved in H_2O (0.5 mL). The reaction mixture was stirred at 25 °C for 3 h, during which time all of the starting material had been consumed to give the desired carboxylic acid as the major product along with two slightly more polar byproducts. Upon solvent removal, the residue left over was diluted with saturated NH_4Cl (1 mL) and treated with 1 M HCl (0.5 mL, pH ≈ 3.0). Following dilution with H_2O (15 mL) and extraction with EtOAc (3 × 15 mL), the combined organic layer was concentrated and the residue obtained passed through a short plug of silica gel (0.7 g) with *n*-hexanes−EtOAc (4:1) to afford carboxylic acid **12** (25 mg, 45% over two steps) as a colorless oil: IR (film) ν_{max} 3028 (br), 2928 (br), 2666, 2549, 1690, 1646, 1495, 1453, 1421, 1286, 1132, 1075, 900, 736, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.30 (2H, t, J = 7.5 Hz), 7.26 (1H, t, J = 7.5 Hz), 7.19 (2H, t, J = 7.5 Hz), 6.95 (1H, t, J = 7.6 Hz), 4.87 (2H, br s), 3.37 (2H, s), 2.84 (2H, d, J = 7.6 Hz), 1.76 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.0, 145.5, 141.9, 138.9, 128.9, 128.4, 128.3, 126.3, 113.1, 43.3, 34.6, 11.9; LRESIMS m/z 215.5 $[M - H]^+$; HREISMS m/z 215.1072 $[M - H]^+$ (calcd for $C_{14}H_{15}O_2$, 215.1072).

Dechloro-smenothiazole A (8). A mixture of **9** (14.3 mg, 0.056 mmol, 1 equiv), carboxylic acid **12** (12.2 mg, 1 equiv), EDC (11.8 mg, 1.1 equiv), and HOBt (8.3 mg, 1.1 equiv) in dry CH_2Cl_2 (3 mL) was stirred at 25 °C for 12 h. The reaction mixture was concentrated to dryness, basified with saturated $NaHCO_3$ (15 mL), extracted with EtOAc (3 × 15 mL), and concentrated under reduced pressure to leave a residue, which was passed through a short plug of silica gel (0.7 g) with $CHCl_3$−*n*-hexanes (9:1) to afford **8** (19.8 mg, 78% yield) as a 1:1 mixture of epimers as a colorless oil: IR (film) ν_{max} 3325, 3082, 3027, 2961, 2929, 1782, 1718, 1635, 1496, 1430, 1321, 1249, 1176, 1076, 897, 738, 701 cm^{-1}; ^{1}H NMR ($CDCl_3$, 700 MHz) δ 7.70/7.67 (1H, d, J = 3.2 Hz, H-1); 7.28 (2H, m, H-23/H-25); 7.23/7.16 (1H, d, J = 3.3 Hz, H-2); 7.20 (1H, m, H-24); 7.17 (2H, m, H-22/H-26); 6.41/6.33 (1H, dt, J = 8.1 Hz, H-16); 6.23/5.93 (NH, d, J = 9.1 Hz); 5.52/5.48 (1H, dd, J = 8.0, 2.8 Hz, H-4); 4.85 (1H, br s, H-19); 4.84 (1H, br s, H-19′); 4.77/4.72 (1H, dd, J = 8.7, 5.8 Hz, H-9); 3.87/3.78 (2H, m, H-7); 3.35 (2H, br s, H-20); 2.78 (2H, d, J = 7.0 Hz, H-17); 2.38 (1H, m, H-10); 2.27/2.10 (2H, m, H-5/H-5′), 2.15/2.04 (2H, m, H-6); 1.76/1.66 (3H, s, H-15); 1.01/0.98 (3H, d, J = 6.7 Hz, H-12); 0.91/0.79 (3H, d, J = 6.7 Hz, H-11); ^{13}C NMR ($CDCl_3$, 176 MHz) δ 171.9/171.4 (C, C-3), 171.7/170.9 (C, C-8), 169.1/169.0 (C, C-13), 145.9/145.8 (C, C-18), 142.3 (CH, C-1), 139.1/139.0 (C, C-21), 133.6/133.3 (CH, C-16), 132.2/132.0 (C, C-14), 128.9 (CH, C-22,26), 128.4 (CH, C-23,25), 126.3/126.2 (CH, C-24), 118.8/118.7 (CH, C-2), 112.9/112.8 (CH_2, C-19), 58.6/58.4 (CH, C-4), 55.9/55.3 (CH, C-9), 47.4/47.2 (CH_2, C-7), 43.2 (CH_2, C-20), 34.3 (CH_2, C-17), 31.9/31.5 (CH_2, C-5), 31.6/31.2 (CH, C-10), 24.5/23.9 (CH_2, C-6), 19.8/19.6 (CH_3, C-12), 18.0/17.5 (CH_3, C-11), 12.7/12.6 (CH_3, C-15); LRESIMS m/z 490.1 $[M + K]^+$, 474.2 $[M + Na]^+$, 452.2 $[M + H]^+$; HREISMS m/z 452.2372 $[M + H]^+$ (calcd for $C_{26}H_{34}N_3O_2S$, 452.2372).

VCD Measurements. The VCD and IR spectra of **6** dissolved in CD_3CN were measured using the ChiralIR-2*X* FT-VCD spectrometer from BioTools Inc. The spectra were collected in the 2000−1000 cm^{-1} range at a resolution of 4 cm^{-1}. The spectrometer was equipped with dual sources and dual ZnSe photoelastic modulators optimized at 1400 cm^{-1}. A solution with a concentration of ca. 0.14 M was measured in a BaF_2 cell with a path length of 99.7 μm. The final spectrum was averaged from 9 blocks, each of 2048 interferometric scans (1 block accumulated for 40 min). Baseline correction using the spectrum of the relevant solvent obtained under the same conditions was performed.

ECD Measurements. The ECD and UV spectra of **6** in MeCN (~1.7×10^{-4} M) were recorded in a quartz cell with a path length of 0.1 cm between 300 and 180 nm at 25 °C on a Jasco J-815 spectrometer. The spectrum was recorded using a 100 nm/min scanning speed, a step size of 0.2 nm, a bandwidth of 1 nm, a response time of 0.5 s, and an accumulation of 10 scans. The spectra were background corrected using respective solvents recorded under the same conditions.

Evaluation of Antituberculosis Activity. In vitro antituberculosis screenings of compounds **1**−**5** and **8** against the pathogenic microbe *Mycobacterium tuberculosis* H_{37}Rv were performed as previously described.[28] Rifampicin was used as positive control during the assay.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00300.

Computational results of **6** (Scheme S1, Figures S1−S9, and Tables S1−S5), Figure S10, Table S6, ^{1}H, ^{13}C, and 2D NMR spectra of **2** and **3**, and underwater photograph of the sponge−sponge consortium (PDF)

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Notes

The authors declare no competing financial interest.

DOI: 10.1021/acs.jnatprod.7b00300
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■ ACKNOWLEDGMENTS

Financial support to C.J.-R. was provided by the IFARHU-SENACYT Program of the Republic of Panama. We thank J. Vicente, J. Asencio, and the crew of the *R/V Sultana* for providing logistic support during collection of the sponge specimens. We are particularly indebted to J. Vicente (University of Maryland Center for Environmental Science) for helpful discussions and for identification of the sponges. Dr. M. Jawiczuk from the Institute of Organic Chemistry, Polish Academy of Sciences, is acknowledged for providing the ECD measurements. The calculations were performed at the Interdisciplinary Centre for Mathematical and Computational Modeling of the University of Warsaw (G19-4) and the PL-Grid Infrastructure. We are grateful to Y. Wang and B. Wan (ITR-UIC) for technical assistance during anti-TB and cytotoxicity assays. This research was supported by the NIH Grant 1SC1GM086271-01A1 awarded to A.D.R.

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(10) We chose **6** over **7** as the best target for chiroptical studies as both its molecular arrangement and specific rotation data matched more closely those of natural plakinidone B (**2**).

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(12) The observation that **1** and **2** have $[\alpha]_D$ values of the same negative sign and similar magnitudes suggests that they share the same absolute configuration of the substituted tetronic acid ring. Thus, our contention is that the specific rotations in **1** and **2** are dominated by the C-17 and C-19 configurations, respectively, and that for **1** the sign appears to be insensitive to the stereocenter remote from the furanone ring. For further validation of these arguments, see refs 1a and 2a.

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(23) Naturally, with compound **8** in hand we were compelled to complete the total synthesis of smenothiazole A (**5**). Unfortunately, multiple attempts to install the vinyl chloride group through cross-metathesis of **8** and *cis*-1,2-dichloroethene using HG-II in C_6D_6 led to degradation of the starting material and formation of intractable mixtures of polar byproducts; see: (a) Jiménez-Romero, C. *Secondary Metabolites from Two Caribbean Sea Hares and the Sponge Association Plakortis-Xestospongia: Isolation, Structure Elucidation, Synthesis, and Biological Evaluation*; Ph.D. Thesis, University of Puerto Rico: San Juan, PR, 2016; pp 180−243. (b) Macnaughtan, M. L.; Gary, J. B.; Gerlach, D. L.; Johnson, M. J. A.; Kampf, J. W. *Organometallics* **2009**, *28*, 2880−2887.

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DOI: 10.1021/acs.jnatprod.7b00300
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