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Article

Scalable Biomimetic Syntheses of Paeciloketal B, 1-*epi*-Paeciloketal B, and Bysspectin A

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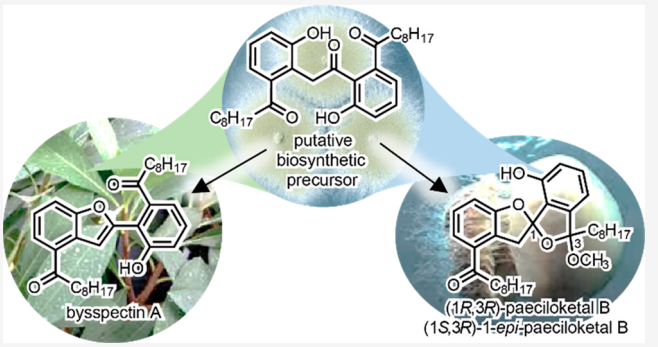
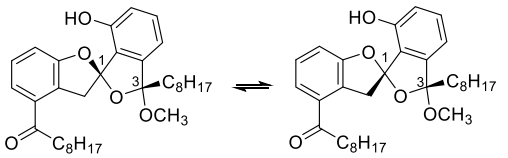
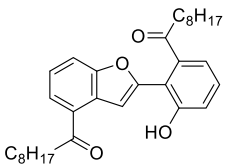
ABSTRACT: The first total synthesis of the benzannulated 5,5-spiroketal natural products paeciloketal B and 1-*epi*-paeciloketal B has been achieved in 10 linear steps employing a biomimetic spiroketalization. This approach also furnished the related natural product bysspectin A from the same putative biosynthetic precursor as the paeciloketals. Alternatively, bysspectin A could be accessed in only six steps using an improved route. This scalable and efficient synthesis affords insight into the biosynthesis of these natural products in nature.



Benzannulated 5,5-spiroketals are relatively rare in nature (<20 reported), and a number have exhibited promising bioactivity, motivating research into their isolation and synthesis.[1−4] The novel benzannulated 5,5-spiroketal paeciloketal B (**1a**) and its epimer 1-*epi*-paeciloketal B (**1b**) were discovered in 2015. These natural products were isolated from the culture broth of *Paecilomyces variotii*, a fungus acquired from the giant jellyfish *Nemopilema nomurai*, which was collected from the South Korean Sea.[5] While the two epimers were separable by HPLC, **1a** was found to rapidly convert to **1b** in MeOH at room temperature to afford an equilibrium mixture of **1a**:**1b** (11:14).[5] The absolute configuration of the C-1 and C-3 stereocenters was assigned through an ECD study.[5] Preliminary antibacterial screening of the mixture of paeciloketals **1a** and **1b** against *Staphylococcus aureus*, *Streptococcus iniae*, and *Vibrio ichthyoenteri* showed no significant activity (MICs > 40 μg mL^{-1}).



paeciloketal B (**1a**) : 1-*epi*-paeciloketal B (**1b**)
11 : 14



bysspectin A (**2**)

The structurally related benzofuran natural product bysspectin A (**2**) was isolated in 2018 from the endophytic fungus *Byssochlamys spectabilis* (the teleomorph of *P. variotii*[6]), obtained from the leaves of *Edgeworthia chrysantha*, a shrub commonly used in Chinese traditional medicine.[7] Bysspectin A (**2**) failed to exhibit antibacterial activity against *Escherichia coli*, *S. aureus*, *Staphylococcus epidermidis*, or *Mycobacterium smegmatis* (MICs > 128 μg mL^{-1}); however, **2** was found to display reversible, selective inhibition of human carboxylesterase 2 (hCE2) (IC_{50} = 2.01 μM) over hCE1 (IC_{50} > 100 μM). As hCE2 is an important intestinal enzyme in the metabolism of certain anticancer drugs such as irinotecan, capecitabine, flutamide, and gemcitabine's prodrug LY2334737, selective hCE2 inhibitors such as **2** hold potential clinical value as combination therapies to improve the efficacy or ameliorate the associated adverse effects of these anticancer chemotherapies.[8]

Bysspectin A (**2**) was proposed to be biosynthetically derived from reactive ketoaldehyde precursor **3**, which is supported by the coisolation of **2** with bysspectin C (**4**), since **4** could also be derived from **3** through benzylic reduction (or **3** could be derived from **4** through benzylic oxidation) (Scheme 1).[7] Alternatively, pinacol dimerization of ketoaldehyde **3** followed by dehydration could forge ketone **5**, which following cyclization and dehydration, would provide by-

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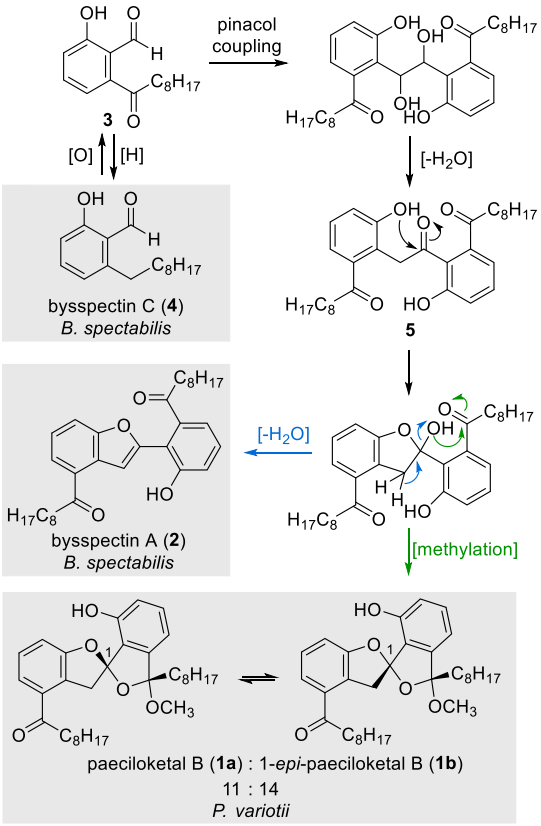
Scheme 1. Plausible Biosynthetic Pathway for Natural Products 1a, 1b, 2, and 4 Isolated from *P. variotii* and *B. spectabilis*[5,7,9]



sspectin A (**2**) (Scheme 1, blue arrows). The similarities in both the structure and natural source of **1a**, **1b**, and **2** make it probable that they share a common biosynthetic pathway; therefore, it is postulated that ketone **5** could alternatively undergo spiroketalization and methylation to provide paeciloketals **1a** and **1b** (Scheme 1, green arrows).

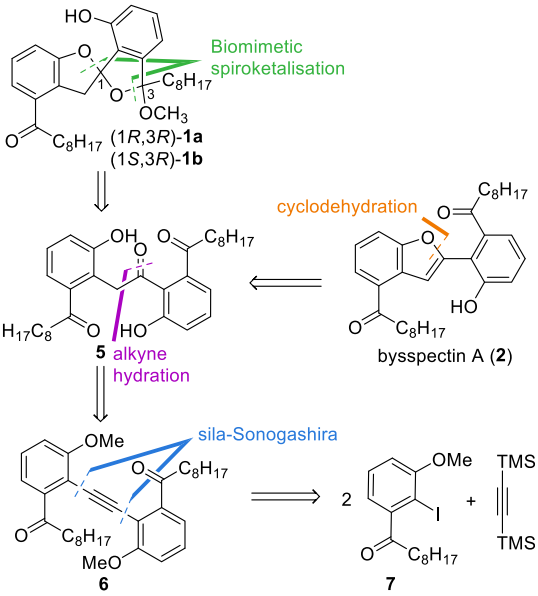
While bysspectin A (**2**) was synthesized in 2019 by Xie and co-workers,[10] paeciloketals **1a** and **1b** have yet to succumb to total synthesis. Owing to our ongoing interest in benzannulated spiroketal natural products[1,2] and biomimetic syntheses,[11−13] we sought to pursue the synthesis of **1a** and **1b**, and in doing so, we hoped to explore their putative biosynthetic relationship to **2**. We anticipated that completion of the synthesis of **1a**, **1b**, and **2** would also enable investigation into the antifungal activity of these compounds, which has not been explored to date.

RESULTS AND DISCUSSION

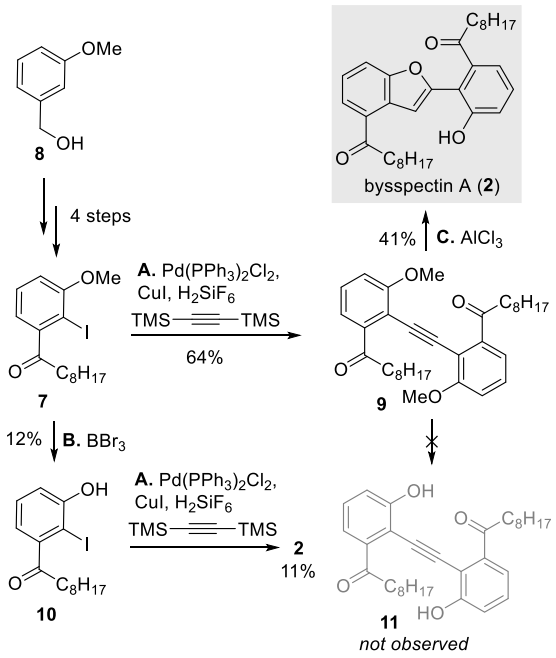
Accordingly, it was decided to target the synthesis of putative biosynthetic precursor **5**, which could be subjected to both biomimetic spiroketalization and methylation (affording **1a** and **1b**) and cyclodehydration (affording **2**), thus providing insight into the proposed biosynthetic pathway (Scheme 2). It was envisaged that ketone **5** could be accessed through hydration and methyl ether cleavage of diarylalkyne **6**, which in turn could be obtained through sila-Sonogashira coupling of known aryl iodide **7**[10] with bis(trimethylsilyl)acetylene.

To this end, aryl iodide **7**[10] was prepared from 3-methoxybenzyl alcohol (**8**) in four steps using literature procedures (Scheme 3) (see Supporting Information for

Scheme 2. Retrosynthesis of Paeciloketals 1a and 1b and Bysspectin A (2)



Scheme 3. Efficient Synthesis of Bysspectin A (2) and Attempted Synthesis of 11



[a]Reagents and conditions: (a) bis(TMS)acetylene (0.6 equiv), $Pd(PPh_3)_2Cl_2$, CuI, H_2SiF_6 (aq), diisopropylamine, 70 °C, 17 h, 64% (**7** → **9**), 11% (**10** → **2**); (b) BBr_3, CH_2Cl_2, −78 °C to rt, 4 h, 12%; (c) $AlCl_3$, chlorobenzene, 75 to 85 to 100 °C, 2 h, 41%.

details).[7,14,15] Pleasingly, it was found that sila-Sonogashira dimerization of **7** with bis(trimethylsilyl)acetylene (0.6 equiv) using Tolnai's procedure[16] yielded diarylalkyne **9** in good yield. Initial attempts to remove the methyl ether protecting groups of **9** using boron tribromide led to a complex mixture. However, treatment of **9** with aluminum trichloride successfully mediated deprotection followed by rapid cyclization, presumably through Lewis-acid-mediated activation of the alkyne, thus affording bysspectin A (**2**) in moderate yield

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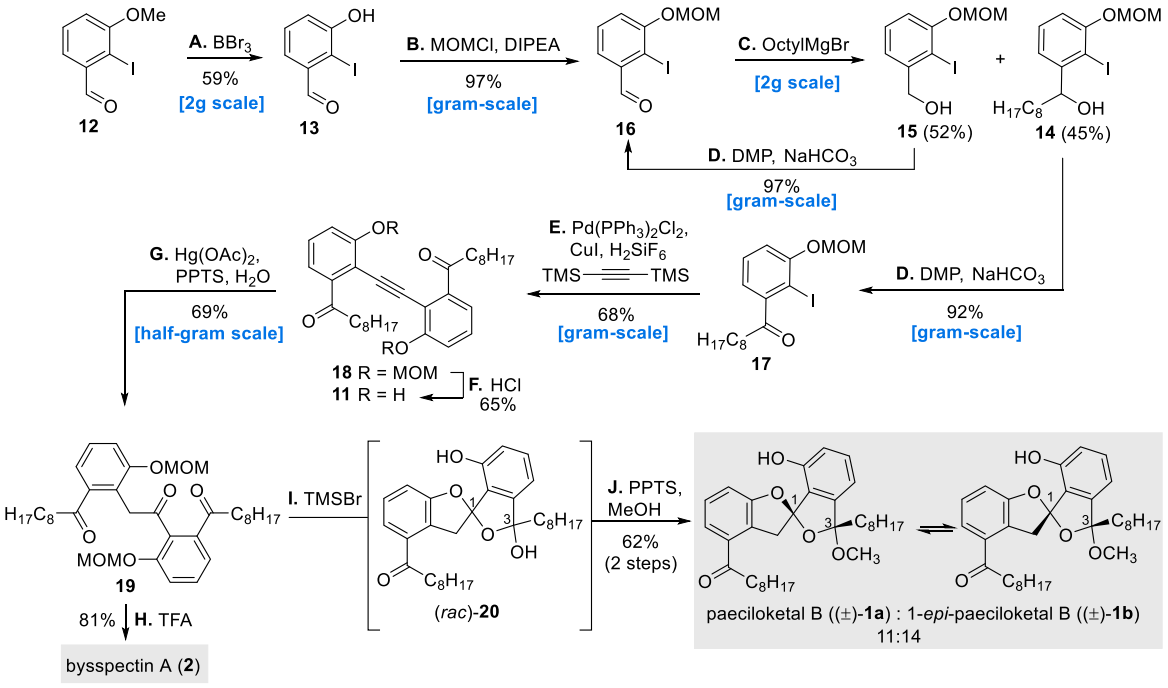
Scheme 4. Scalable Biomimetic Synthesis of Paeciloketals (±)-1a and (±)-1b and Byspectin A (2)



[a]Reagents and conditions: (a) BBr_3, CH_2Cl_2, 0 °C to rt, 4 h, 59%; (b) MOMCl, DIPEA, CH_2Cl_2, 0 °C to rt, 1 h, 97%; (c) OctylMgBr, THF, 0 °C, 30 min, **14** (45%), **15** (52%); (d) DMP, $NaHCO_3$, CH_2Cl_2, rt, 1 h, 97% for **16**, 92% for **17**; (e) bis(TMS)acetylene (0.6 equiv), $Pd(PPh_3)_2Cl_2$, CuI, H_2SiF_6 (aq), diisopropylamine, 80 °C, 65 h, 68%; (f) HCl, MeOH, CH_2Cl_2, rt, 4 h, 65%; (g) $Hg(OAc)_2$, PPTS, H_2O, THF, 45 °C, 1.5 h, 69%; (h) TFA, CH_2Cl_2, rt, 1.5 h, 81%; (i) TMSBr, CH_2Cl_2, −10 °C, 20 min; (j) PPTS, MeOH, CH_2Cl_2, 0 °C, 40 min, 62% over 2 steps.

(41%, unoptimized). Bysspectin A (**2**) has been previously synthesized using a convergent route (13 steps total) with an overall yield of 7.7%;[10] however, this route represents a more step-efficient synthesis of the natural product, requiring only 6 steps to access **2** in 5.8% overall yield.

While this result was a welcome digression, our attention returned to the primary objective: biomimetic synthesis of paeciloketals **1a** and **b** from ketone **5**. As the methyl ether cleavage of iodide **7** using boron tribromide had previously been reported by Xie and co-workers in their synthesis of bysspectin A (**2**), this was attempted next.[10] In our hands, however, phenol **10** was obtained in poor yield (12%), in contrast with the reported yield of 74%.[10] Nevertheless, sufficient material was obtained to attempt the sila-Sonogashira coupling. Frustratingly, under the aforementioned conditions, considerable degradation occurred, and only bysspectin A (**2**) was isolated in poor yield (11%), indicating that desired product **11** was prone to cyclization under the reaction conditions and that this approach was unlikely to allow access to ketone **5**.

A more labile protecting group was deemed necessary to allow for late stage removal under milder conditions. Methoxymethyl (MOM) ether was chosen, since Türkmen and Rawal have demonstrated that diphenylacetylene diols can be prepared in excellent yield through deprotection of the corresponding MOM ethers with hydrochloric acid at room temperature.[17] Deprotection of known benzaldehyde **12**[15] (previously prepared en route to **7**) with boron tribromide according to the procedure described by Kingston and co-workers[18] afforded desired phenol **13** in good yield (2 g scale, Scheme 4). MOM protection of phenol **13** (gram scale), followed by a Grignard reaction with octylmagnesium bromide (2 g scale), afforded desired alcohol **14** in moderate yield alongside reduced benzyl alcohol **15**. Nevertheless, benzyl alcohol **15** could be recycled back to benzaldehyde **16** through oxidation with Dess-Martin periodinane (DMP), thus providing alcohol **14** in 68% overall yield after one round of recycling. Oxidation of **14** provided ketone **17**, which underwent gram-scale sila-Sonogashira dimerization with bis(trimethylsilyl)acetylene using the previously identified conditions to provide diarylalkyne **18** in 68% yield. Pleasingly, **18** then underwent MOM ether cleavage smoothly with hydrochloric acid to afford **11** in good yield. With **11** in hand, hydration of the alkyne could next be investigated. Hamze and co-workers reported the use of PtO_2 and *p*-toluenesulfonic acid (*p*TSA) in aqueous MeOH for the internal hydration of diarylalkynes and stated that omission of PtO_2 was required for substrates bearing *ortho*-heteroatoms to avoid indole/benzofuran formation.[19] It was thought that subjecting **11** to these conditions might not only effect hydration of the alkyne but also mediate concomitant spiroketalization and MeOH addition to the resulting hemiketal under the acidic methanolic conditions to provide **1a** and **1b** in a one-pot biomimetic cascade.[20] Disappointingly, however, under these conditions, **11** was only observed to form bysspectin A (**2**), indicating that the alkyne was more prone to attack from the adjacent phenols than from H_2O, even in the absence of PtO_2. MOM-protected diarylalkyne **18** was also subjected to these conditions (PtO_2, *p*TSA, MeOH, H_2O, 70 → 90 °C); however, this led to formation of a complex mixture suspected to result from partial deprotection of the MOM ethers. Thus, it was thought that milder conditions might be required to promote hydration of **18** without mediating deprotection and subsequent formation of **2**. While both sodium sulfide/hydrochloric acid in aqueous MeOH[21] and $PdCl_2$ in aqueous dioxane[22] promoted only MOM ether cleavage and degradation respectively, treatment

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of **18** with $Hg(OAc)_2$ and pyridinium *p*-toluenesulfonate (PPTS) in aqueous THF[23] pleasingly afforded desired ketone **19** in good yield on a half-gram scale (460 mg) with no observable formation of **2**. Ketone **19** was selected as the end point of the scalable synthesis, since this compound serves as a divergent point for exploration of the biomimetic transformations of **19** into both paeciloketals **1a**/**b** and bysspectin A (**2**). Thus, with ample quantities of **19** in hand, the final MOM deprotection, spiroketalization, and methylation sequence toward paeciloketals **1a** and **1b** could be attempted.

Again, it was thought that acidic methanolic conditions could feasibly achieve this sequence in a biomimetic cascade.[20] Unfortunately, treating **19** with either aqueous hydrochloric acid or *p*TSA in $MeOH/CH_2Cl_2$ (1:1) led to formation of complex mixtures. Interestingly, however, TFA was found to mediate clean conversion of **19** to bysspectin A (**2**) in excellent yield (81%), thus supporting the proposed biosynthetic cyclodehydration of **5** to form **2** in nature. To our delight, bromotrimethylsilane[24] smoothly effected deprotection and spiroketalization of **19** in one pot, to provide hemiketal (*rac*)-**20** (as a 1:1 mixture of diastereomers). Interestingly, (*rac*)-**20** was prone to hemiketal ring opening and dehydration to form **2** (as observed by TLC) either when the reaction was quenched with brine or when the crude material was concentrated in the presence of residual HBr, thus lending further evidence to support the common biosynthetic pathway of **1a**/**b** and **2** in nature. However, careful neutralization of the reaction with 50% aqueous sodium bicarbonate provided (*rac*)-**20**, which could be isolated; however, owing to its observed instability upon storage, the crude material was treated directly with methanolic PPTS to provide paeciloketals (±)-**1a** and (±)-**1b** in 62% yield over two steps. The diastereomeric ratio (**1a**:**1b**, 11:14) along with the ^1H and ^{13}C NMR data of synthetic (±)-**1a** and (±)-**1b** showed excellent agreement with that reported for the natural products;[5] however, careful analysis of the 2D NMR data of synthetic (±)-**1a**/**b** led us to review the assignments for several of the signals (see Supporting Information for details). The authors of the isolation paper reportedly detected **1a** and **1b** in MeOH-free extracts of the culture broth by both normal-phase and reversed-phase TLC analysis.[5] While TLC analysis of an extract containing a complex mixture of metabolites is not a conclusive method of unequivocally confirming the presence of methyl ketals **1a** and **1b** in the MeOH-free extract, the observed facile dehydration of hemiketal (*rac*)-**20** in this study corroborates the postulate that the C-3 methyl ketal of **1a**/**b** occurs naturally and is not an artificial derivative of **20**. However, in the absence of conclusive evidence, it remains plausible that **1a** and **1b** are isolation artifacts resulting from reaction of hemiketal **20** with MeOH.

The authors of the isolation paper reported that **1a** and **1b** existed as an 11:14 mixture of epimers (1*R*,3*R*/1*S*,3*R*) that were prone to epimerization at the spiroketal C-1 stereocenter but that no epimerization was observed at C-3. Since we observed that putative biosynthetic intermediate hemiketal **20** is prone to ring opening and dehydration to form bysspectin A (**2**), this indicates that C-3 of hemiketal **20** would also be subject to epimerization but that the methyl ketal (of **1a** and **1b**) stabilizes this center and prevents C-3 epimerization. Taken together, this suggests that in nature enzymatic control is required to set the methyl ketal C-3*R* stereoconfiguration of **1a** and **1b**. Furthermore, as **1a** and **1b** were characterized as a mixture, and both the C-1 and C-3 stereocenters of **20** were thought to be prone to epimerization, attempting an asymmetric spiroketalization of **19** using chiral acids was expected to be unproductive.

Natural products (±)-**1a**/**b** and **2** were evaluated for antifungal activity using minimum inhibitory concentration (MIC) assays against two species of yeast, *Candida albicans* SC5314 (type strain) and *Candida utiliz* SVB-Y1 (clinical isolate), and one species of mold, *Aspergillus fumigatus* SVB-F136 (clinical isolate). Unfortunately, both compounds failed to show inhibitory activity toward any of the tested strains (MIC > 64 μM, Table S1, Supporting Information).

In summary, a scalable and efficient first synthetic route to paeciloketals (±)-**1a** and (±)-**1b** was established in 6.1% yield over 10 steps using a biomimetic spiroketalization of ketone **19**. Bysspectin A (**2**) was prepared in only six linear steps, a much more efficient route than that previously disclosed.[10] The facile formation of **2** from putative biosynthetic precursors **19** and **20** under several conditions lends evidence to support the proposed biosynthetic pathway, and the successful synthesis of (±)-**1a** and (±)-**1b** from putative biosynthetic precursor **19** supports their proposed biosynthetic relationship to bysspectin A (**2**). Additionally, observations on the stability of hemiketal (*rac*)-**20** and natural products **1a** and **1b** suggest enzymatic catalysis is required for the selective formation of the C-3*R* methyl ketal stereoconfiguration of the natural products in nature.

EXPERIMENTAL SECTION

General Experimental Procedures. Infrared spectra were obtained using a PerkinElmer spectrum One Fourier Transform Infrared spectrometer. Absorption maxima are expressed in wavenumbers (cm^{-1}). NMR spectra were recorded as indicated on either a Bruker 400 MHz Avance III spectrometer or a Bruker 500 MHz Avance III-HD spectrometer (equipped with a cryogenically cooled probehead) as specified. ^1H NMR chemical shifts are reported in parts per million (ppm) relative to the chloroform (δ_H 7.26) or methanol signal (δ_H 3.31), except for the paeciloketals (±)-**1a** and (±)-**1b** ^1H NMR spectrum, which was calibrated to the OCH_3 signal for 1-*epi*-paeciloketal B ((±)-**1b**, δ_H 3.12). The ^{13}C NMR values were referenced to the residual chloroform (δ_C 77.16) or methanol (δ_C 49.0) signal except for the paeciloketals (±)-**1a** and (±)-**1b** ^{13}C NMR spectrum, which was calibrated to the $CH_2(1')$ signal for 1-*epi*-paeciloketal B ((±)-**1b**, δ_C 40.9). NMR assignments were made with the aid of DEPT 90, DEPT 135, COSY, and HSQC experiments. All experiments were conducted at 298 K. Conventional NMR tubes (5 mm diameter, Wilmad) using a sample volume of 500 μL were used. High-resolution mass spectra were obtained by electrospray ionization in positive ion mode at a nominal accelerating voltage of 70 eV on a microTOF mass spectrometer, except for phenol **13**, which required negative ion mode. Analytical thin layer chromatography was performed using silica plates, and compounds were visualized at 254 and/or 365 nm ultraviolet irradiation followed by staining with either alkaline permanganate or ethanolic vanillin solution. Commercially available reagents were used throughout without purification unless otherwise stated. Anhydrous solvents were used as supplied. Tetrahydrofuran and CH_2Cl_2 were dried using an LC Technology Solutions Inc. SP-1 solvent purification system under an atmosphere of dry nitrogen. All reactions were routinely carried out in oven-dried glassware under a nitrogen atmosphere unless otherwise stated. All reactions requiring heating were done so using IKA magnetic hot plates with integrated temperature control sensors and DrySyn heating blocks or sand baths.

General Procedure A: Sila-Sonogashira Coupling. A stirred solution of aryl iodide in diisopropylamine (0.14 M) was degassed with argon by sparging for 10 min, before $Pd(PPh_3)_2Cl_2$ (5 mol %), copper(I) iodide (5 mol %), hexafluorosilicic acid (34% aqueous, 5 μL, 0.048 mmol), and bis(trimethylsilyl)acetylene (0.6 equiv) were

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added in that order. The vessel was sealed under argon and heated to the temperature stated for the specified time. The mixture was then diluted with EtOAc and quenched with H_2O, the layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were then dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel, eluted with petroleum ether−ethyl acetate (4:1) to afford the desired product.

General Procedure B: Dess-Martin Periodinane (DMP) Oxidation. To a stirred solution of alcohol starting material in CH_2Cl_2 (0.064 M) was added sodium bicarbonate (6.8 equiv) followed by DMP (1.7 equiv), and the resulting mixture was stirred at room temperature (rt) for 1 h. Upon complete consumption of the starting material, saturated solutions of sodium bicarbonate (20 mL) and sodium thiosulfate (20 mL) were added, and the mixture was stirred vigorously for 10 min. The layers were then separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel, eluted with petroleum ether−EtOAc (9:1) to afford the desired product.

Diarylalkyne 9. Aryl iodide **7**[10] (20 mg, 0.0535 mmol) was subjected to General Procedure A, with heating to 70 °C in a screw-capped vial for 17 h to afford diarylalkyne **9** (8.8 mg, 0.0170 mmol, 64%) as an orange waxy solid. IR ν_{max}/cm^{-1} (neat): 2922, 2851, 1689, 1568, 1466, 1270, 1294, 997, 787, 737; δ_H (400 MHz, $CDCl_3$) 7.33 (2H, t, *J* 8.1, 2 × CH), 7.10 (2H, dd, *J* 7.5, 1.0, 2 × CH), 6.98 (2H, d, *J* 8.0, 2 × CH), 3.91 (6H, s, 2 × OCH_3), 3.19 (4H, t, *J* 7.5, 2 × CH_2), 1.68 (4H, pent, *J* 7.5, 2 × CH_2), 1.32−1.20 (20H, m, 10 × CH_2), 0.84 (6H, t, *J* 7.0, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 205.3 (2 × C), 160.8 (2 × C), 144.5 (2 × C), 129.7 (2 × CH), 119.7 (2 × CH), 112.6 (2 × CH), 110.2 (2 × C), 93.6 (2 × C), 56.2 (2 × OCH_3), 42.8 (2 × CH_2), 32.0 (2 × CH_2), 29.6 (2 × CH_2), 29.5 (2 × CH_2), 29.3 (2 × CH_2), 24.7 (2 × CH_2), 22.8 (2 × CH_2), 14.2 (2 × CH_3); HRMSESIMS *m/z*: 541.3273 $[M + Na]^+$ (calcd for $C_{34}H_{46}O_4Na$, 541.3288); R_f 0.33 (petroleum ether−EtOAc, 4:1).

Bysspectin A (2). *Method A.* To a stirred solution of diarylalkyne **9** (18 mg, 0.0347 mmol) in chlorobenzene (1.4 mL) was added aluminum trichloride (23 mg, 0.135 mmol), and the resulting solution was heated to 75 °C for 30 min, then 85 °C for 30 min, and then 100 °C for 1 h. Upon completion, the reaction mixture was cooled to rt, diluted with EtOAc (10 mL), and quenched with H_2O/saturated aqueous sodium hydrogen carbonate (1:1, 10 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (10 mL). The combined organic layers were dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel, eluted with petroleum ether−EtOAc (85:15) to afford bysspectin A (**2**) (7.0 mg, 0.0143 mmol, 41%) as a yellow oil.

Method B. To a stirred solution of **19** (10 mg, 0.0168 mmol) in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (0.1 mL), and the resulting solution was stirred at rt for 1.5 h. Upon complete consumption of the starting material, the reaction was diluted with CH_2Cl_2 (10 mL) and washed with H_2O (5 mL). The organic layer was dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel, eluted with petroleum ether−EtOAc (4:1) to afford bysspectin A (**2**) (6.7 mg, 0.0137 mmol, 81%) as a yellow oil. ν_{max}/cm^{-1} (neat): 3357, 2923, 2854, 1678, 1587, 1456, 1424, 1289, 1259, 921, 793, 746; HRMSESIMS *m/z*: 513.2969 $[M + Na]^+$ (calcd for $C_{32}H_{42}O_4Na$, 513.2975); R_f 0.41 (petroleum ether−EtOAc, 7:3). ^1H and ^{13}C NMR, Tables S2 and S3 (Supporting Information).

3-Hydroxy-2-iodobenzaldehyde (13). To a stirred solution of aryl iodide **12**[15] (2.68 g, 10.2 mmol) in CH_2Cl_2 (100 mL) at 0 °C was added boron tribromide (1 M solution in CH_2Cl_2, 25.5 mL, 25.5 mmol) dropwise, and the resulting solution was warmed to rt for 4 h. The solution was then carefully quenched with H_2O (20 mL) and diluted with CH_2Cl_2 (50 mL). The layers were separated, and the organic layer was washed with brine (50 mL), dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel, eluted with petroleum ether−EtOAc (3:1) to afford phenol **13** (1.49 g, 6.01 mmol, 59%) as a pale yellow amorphous solid. ν_{max}/cm^{-1} (neat): 3128, 1660, 1562, 1455, 1290, 1274, 1252, 1216, 1164, 1015, 780; δ_H (400 MHz, $CDCl_3$) 10.03 (1H, s, CHO), 7.46 (1H, dd, *J* 7.5, 1.5, CH), 7.36 (1H, t, *J* 7.8, CH), 7.27 (1H, dd, *J* 8.0, 2.0, CH), 5.80 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 194.7 (CHO), 155.6 (C), 135.9 (C), 130.1 (CH), 124.2 (CH), 120.9 (CH), 91.2 (C); HRMSESIMS *m/z*: 246.9263 $[M - H]^-$ (calcd for $C_7H_4IO_2$, 246.9262); R_f 0.25 (petroleum ether−EtOAc, 3:1).

2-Iodo-3-(methoxymethoxy)benzaldehyde (16). To a stirred solution of phenol **13** (1.49 mg, 6.02 mmol) in CH_2Cl_2 (65 mL) at 0 °C was added diisopropylethylamine (2.02 mL, 11.6 mmol) and chloromethyl methyl ether (0.67 mL, 7.85 mmol). The resulting solution was warmed to rt and stirred for 1 h, before being diluted with CH_2Cl_2 (50 mL) and quenched with H_2O (50 mL). The layers were separated, and the organic layer was washed with H_2O/saturated aqueous sodium hydrogen carbonate (1:1, 60 mL). The combined organic layers were then dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel, eluted with petroleum ether−EtOAc (9:1) to afford **16** (1.71 g, 5.86 mmol, 97%) as a colorless amorphous solid. ν_{max}/cm^{-1} (neat): 3079, 2893, 1688, 1563, 1457, 1385, 1257, 1233, 1155, 1083, 1001, 921, 893, 785; δ_H (400 MHz, $CDCl_3$) 10.19 (1H, s, CHO), 7.55 (1H, dd, *J* 7.5, 1.5, CH), 7.36 (1H, td, *J* 7.8, 1.0, CH), 7.31 (1H, dd, *J* 8.1, 1.6, CH), 5.30 (2H, s, CH_2), 3.54 (3H, s, OCH_3); δ_C (100 MHz, $CDCl_3$) 196.4 (CHO), 156.5 (C), 137.0 (C), 129.6 (CH), 123.7 (CH), 120.2 (CH), 95.4 (CH_2), 94.9 (C), 56.7 (OCH_3); HRMSESIMS *m/z*: 314.9485 $[M + Na]^+$ (calcd for $C_9H_9IO_3Na$, 314.9489); R_f 0.43 (petroleum ether−EtOAc, 4:1).

Benzyl Alcohol 15 and Alcohol 14. A two-necked flask attached with a Vigreux condenser and charged with magnesium granules (768 mg, 31.6 mmol) was flame-dried and then allowed to cool under vacuum. The apparatus was flushed with argon, and then, enough THF was added to just cover the magnesium (∼5 mL). Dibromoethane (∼0.1 mL) was then added, followed by trimethylsilyl chloride (∼0.1 mL) until the mixture was bubbling vigorously and the flask was hot to the touch, at which point 1-bromooctane (4.42 mL, 25.6 mmol) was added dropwise as a solution in THF (25 mL). The resulting mixture was heated to reflux for 1.5 h under argon and then left to cool to rt without stirring for 1 h to allow the residual magnesium to settle.

To a stirred solution of benzaldehyde **16** (2.002 g, 6.86 mmol) in THF (80 mL) at 0 °C was added the octylmagnesium bromide solution (12 mL, estimated 8.93 mmol) dropwise, and the resulting solution was stirred for 30 min. Upon complete consumption of the starting material, the reaction mixture was quenched with saturated aqueous ammonium chloride (40 mL), and the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were then dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel, eluted with petroleum ether−EtOAc (4:1) to afford the desired alcohol **14** (1.25 g, 3.07 mmol, 45%) as a colorless waxy solid, alongside benzyl alcohol **15** (1.06 g, 3.59 mmol, 52%) as a colorless amorphous solid. Benzyl alcohol **15** (1.05 g, 3.57 mmol) could be recycled back to benzaldehyde **16** (1.01 g, 3.46 mmol, 97%) according to General Procedure B, thus providing desired alcohol **14** in 68% overall yield after 1 round of recycling. **Alcohol 14**: ν_{max}/cm^{-1} (neat): 3389, 2923, 2853, 1458, 1252, 1153, 1007, 923, 786, 717; δ_H (400 MHz, $CDCl_3$) 7.28 (1H, t, *J* 8.0, CH), 7.18 (1H, dd, *J* 7.6, 1.5, CH), 6.97 (1H, dd, *J* 8.2, 1.5, CH), 5.26, 5.24 (2H, ABq, *J* 6.5, CH_2), 5.03−5.00 (1H, m, CH), 3.52 (3H, s, OCH_3), 1.96 (1H, brs, OH), 1.81−1.74 (1H, m, 1/2 × CH_2), 1.66−1.56 (1H, m, 1/2 × CH_2), 1.53−1.27 (12H, m, 6 × CH_2), 0.88 (3H, t, *J* 6.7, CH_3); δ_C (100 MHz, $CDCl_3$) 156.7 (C), 149.1 (C), 129.5 (CH), 120.5 (CH), 113.8 (CH), 95.3 (CH_2), 91.6 (C), 77.8 (CH), 56.6 (OCH_3), 37.9 (CH_2), 32.0 (CH_2), 29.68 (CH_2), 29.63 (CH_2), 29.4 (CH_2), 26.1 (CH_2), 22.8 (CH_2), 14.3 (CH_3); HRMSESIMS *m/z*: 429.0890 $[M + Na]^+$ (calcd for $C_{17}H_{27}IO_3Na$, 429.0897); R_f 0.31 (petroleum ether−EtOAc, 17:3). **Benzyl alcohol 15**: ν_{max}/cm^{-1} (neat): 3289, 2899,

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1459, 1253, 1147, 1003, 918, 766; δ_H (400 MHz, $CDCl_3$) 7.29 (1H, t, *J* 8.0, CH), 7.13 (1H, dd, *J* 7.3, 1.2, CH), 7.01 (1H, dd, *J* 8.0, 1.2, CH), 5.26 (2H, s, CH_2), 4.72 (2H, d, *J* 6.5, CH_2), 3.52 (3H, s, OCH_3), 2.04 (1H, t, *J* 6.5, OH); δ_C (100 MHz, $CDCl_3$) 156.0 (C), 144.8 (C), 129.5 (CH), 122.1 (CH), 114.1 (CH), 95.2 (CH_2), 90.8 (C), 69.9 (CH_2), 56.6 (OCH_3); HRMSESIMS *m/z*: 316.9638 $[M + Na]^+$ (calcd for $C_9H_{11}IO_3Na$, 316.9645); R_f 0.13 (petroleum ether−EtOAc, 17:3).

1-(2-Iodo-3-(methoxymethoxy)phenyl)nonan-1-one (17). Carbonyl **17** (1.14 g, 2.82 mmol, 92%) was prepared as a yellow oil from alcohol **14** (1.25 g, 3.08 mmol) according to General Procedure B. ν_{max}/cm^{-1} (neat): 2924, 2854, 1701, 1563, 1457, 1421, 1255, 1154, 973, 923, 782; δ_H (400 MHz, $CDCl_3$) 7.31 (1H, dd, *J* 8.5, 7.5, CH), 7.09 (1H, dd, *J* 8.5, 1.5, CH), 6.88 (1H, dd, *J* 7.5, 1.5, CH), 5.26 (2H, s, CH_2), 3.52 (3H, s, OCH_3), 2.86 (2H, t, *J* 7.3, CH_2), 1.72 (2H, pent, *J* 7.4, CH_2), 1.39−1.27 (10H, m, 5 × CH_2), 0.88 (3H, t, *J* 7.0, CH_3); δ_C (100 MHz, $CDCl_3$) 206.3 (C), 156.4 (C), 148.6 (C), 129.7 (CH), 120.4 (CH), 115.6 (CH), 95.2 (CH_2), 84.4 (C), 56.7 (OCH_3), 42.8 (CH_2), 32.0 (CH_2), 29.5 (CH_2), 29.32 (CH_2), 29.28 (CH_2), 24.1 (CH_2), 22.8 (CH_2), 14.2 (CH_3); HRMSESIMS *m/z*: 427.0731 $[M + Na]^+$ (calcd for $C_{17}H_{25}IO_3Na$, 427.0741); R_f 0.44 (petroleum ether−EtOAc, 17:3).

Diarylalkyne 18. Aryl iodide **17** (1.14 g, 2.82 mmol) was subjected to General Procedure A, with heating to 80 °C in a sealed tube for 65 h to afford diarylalkyne **18** (557 mg, 0.963 mmol, 68%) as a yellow amorphous solid. ν_{max}/cm^{-1} (neat): 2919, 2853, 1691, 1568, 1464, 1249, 1150, 1037, 977, 788, 735; δ_H (400 MHz, $CDCl_3$) 7.32 (2H, dd, *J* 8.5, 7.5, 2 × CH), 7.25 (2H, dd, *J* 1.0, 2 × CH)*, 7.15 (2H, dd, *J* 7.5, 1.5, 2 × CH), 5.27 (4H, s, 2 × CH_2), 3.51 (6H, s, 2 × OCH_3), 3.14 (4H, t, *J* 7.4, 2 × CH_2), 1.67 (4H, pent, *J* 7.4, 2 × CH_2), 1.31−1.20 (20H, m, 10 × CH_2), 0.85 (6H, t, *J* 7.0, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 204.9 (2 × C), 158.7 (2 × C), 144.5 (2 × C), 129.7 (2 × CH), 120.9 (2 × CH), 117.1 (2 × CH), 111.3 (2 × C), 95.2 (2 × CH_2), 93.1 (2 × C), 56.5 (2 × OCH_3), 42.7 (2 × CH_2), 31.9 (2 × CH_2), 29.6 (2 × CH_2), 29.5 (2 × CH_2), 29.3 (2 × CH_2), 24.8 (2 × CH_2), 22.8 (2 × CH_2), 14.2 (2 × CH_3), *Signal partially occluded by residual $CHCl_3$ peak; HRMSESIMS *m/z*: 601.3477 $[M + Na]^+$ (calcd for $C_{36}H_{50}O_6Na$, 601.3500); R_f 0.26 (petroleum ether−EtOAc 4:1).

Ketone 19. To a stirred solution of diarylalkyne **18** (464 mg, 0.802 mmol) in THF (19 mL) was added pyridinium *p*-toluenesulfonate (305 mg, 1.21 mmol) and H_2O (0.16 mL), followed by mercury(II) acetate (80 mg, 0.251 mmol, 30 mol %), and the resulting mixture was heated to 45 °C for 1.5 h. The mixture was then cooled to rt, diluted with EtOAc (30 mL), and washed with H_2O/saturated aqueous sodium hydrogen carbonate (1:1, 40 mL). The organic layer was then dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel, eluted with petroleum ether−EtOAc (4:1) to afford ketone **19** (332 mg, 0.557 mmol, 69%) as a yellow amorphous solid. ν_{max}/cm^{-1} (neat): 2924, 2854, 1687, 1578, 1456, 1258, 1152, 984, 922, 734; δ_H (400 MHz, $CDCl_3$) 7.39−7.37 (2H, m, 2 × CH), 7.36−7.32 (1H, m, CH), 7.29−7.27 (2H, m, 2 × CH), 7.19 (1H, dd, *J* 5.3, 3.6, CH), 5.22 (2H, s, CH_2), 5.21 (2H, s, CH_2), 4.59 (2H, s, CH_2), 3.51 (3H, s, OCH_3), 3.49 (3H, s, OCH_3), 2.96 (2H, t, *J* 7.6, CH_2), 2.81 (2H, t, *J* 7.5, CH_2), 1.71 (2H, pent, *J* 7.4, CH_2), 1.61 (2H, pent, *J* 7.4, CH_2), 1.37−1.26 (20H, m, 10 × CH_2), 0.89−0.86 (6H, m, 2 × CH_3); δ_C (100 MHz, $CDCl_3$) 206.2 (C), 201.7 (C), 200.7 (C), 156.8 (C), 154.5 (C), 142.9 (C), 138.0 (C), 132.3 (C), 130.2 (CH), 127.8 (CH), 121.85 (CH), 121.76 (C), 120.6 (CH), 118.6 (CH), 116.7 (CH), 95.3 (CH_2), 94.8 (CH_2), 56.5 (OCH_3), 56.1 (OCH_3), 42.3 (CH_2), 40.5 (CH_2), 39.8 (CH_2), 32.03 (CH_2), 31.99 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.43 (CH_2), 29.40 (CH_2), 29.3 (CH_2), 24.2 (CH_2), 24.1 (CH_2), 22.82 (CH_2), 22.80 (CH_2), 14.2 (2 × CH_3); HRMSESIMS *m/z*: 619.3599 $[M + Na]^+$ (calcd for $C_{36}H_{52}O_7Na$, 619.3605); R_f 0.23 (petroleum ether−EtOAc, 4:1).

Paeciloketal B and 1-*epi*-Paeciloketal B (11:14, (±)-1a:1b). To a stirred solution of ketone **19** (5 mg, 0.00838 mmol) in CH_2Cl_2 (0.33 mL) at −10 °C was added bromotrimethylsilane (9 μL, 0.0682 mmol), and the resulting solution was stirred for 20 min before being quenched with water (0.2 mL) followed by H_2O/saturated aqueous sodium hydrogen carbonate (1:1, 3 mL). The mixture was diluted with CH_2Cl_2 (5 mL), and the layers were separated. The organic layer was washed with H_2O/saturated aqueous sodium hydrogen carbonate (1:1, 5 mL) and then dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude hemiketal intermediate (*rac*)-**20** could be used directly in the next step or, for characterization purposes, was purified by flash chromatography on silica gel, eluted with petroleum ether−EtOAc (4:1) (78% yield) (see Supporting Information for characterization data). The crude hemiketal intermediate (*rac*)-**20** was dissolved in a solution of MeOH−CH_2Cl_2 (1:1, 1 mL) and cooled to 0 °C, and then, pyridinium *p*-toluenesulfonate (1.5 mg, 0.00597 mmol) was added, and the solution was stirred for 40 min. The solvent was removed under a gentle stream of nitrogen, and then, the crude material was taken up in CH_2Cl_2 (10 mL) and washed with H_2O (5 mL). The organic layer was dried using anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel, eluted with petroleum ether−EtOAc (9:1) to afford an ∼11:14 mixture of (±)-**1a** and (±)-**1b** (3.3 mg, 0.00632 mmol, 62% over two steps) as a pale yellow amorphous solid. ν_{max}/cm^{-1} (neat): 3364, 2922, 2852, 1683, 1609, 1588, 1450, 1353, 1294, 1260, 1155, 1091, 1025, 885, 797, 748; HRMSESIMS *m/z*: 545.3224 $[M + Na]^+$ (calcd for $C_{33}H_{46}O_5Na$, 545.3237); R_f 0.38 and 0.41 (petroleum ether−EtOAc, 4:1). ^1H and ^{13}C NMR, Tables S4−S7.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.1c00502.

Experimental details and characterization data for the synthesis of **7** (from **8** via **12**), **10**, **11**, and (±)-**20a/b**; experimental details and results of the antifungal assays of **2** and (±)-**1a/b**; ^1H and ^{13}C NMR comparisons of synthetic bysspectin A (**2**), (±)-paeciloketal B (**1a**), and (±)-1-*epi*-paeciloketal B (**1b**) with that detailed in their respective isolation reports; ^1H and ^{13}C NMR spectra for compounds **9**, **2**, **13**, **16**, **14**, **15**, **17**, **18**, **11**, **19**, **20**, (±)-**21a/b**, (±)-**1a/b**; COSY and edited HSQC NMR spectra for (±)-**1a/b** (PDF)

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Notes

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