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Syntheses of the Fungal Metabolites Boletopsins 7, 11, and 12 from the Papua New Guinea Medicinal Mushroom *Boletopsis* sp.

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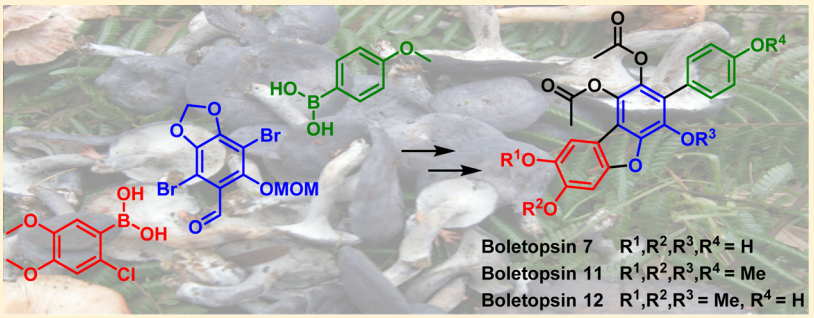
Supporting Information



ABSTRACT: Boletopsins 7 (**1**), 11 (**2**), and 12 (**3**) are *p*-terphenyl dibenzofuran compounds, isolated from the Papua New Guinean medicinal mushroom *Boletopsis* sp. The first syntheses of these fungal metabolites are reported, allowing for an investigation of their antibiotic activity. The key steps include sequential Suzuki−Miyaura couplings to rapidly form the *p*-terphenyl backbone and an Ullmann ether synthesis on a formate ester to create the dibenzofuran moiety. Biological evaluation of the synthetic compounds and intermediates against a panel of bacterial nosocomial pathogens was performed.

INTRODUCTION

Natural products as drugs are an important part of the pharmaceutical industry.[1] The role that fungal metabolites play in the pharmaceutical industry is why natural products are still so relevant, with drug classes such as the statins and cephalosporins contributing to the library.[2] However, the search for new pharmaceuticals faces difficulties, due to the enormous chemical space that the newly found structures consume, making the challenge of finding a viable lead seem overwhelming.

The use of mushrooms in traditional medicine is widespread and well established in traditional Chinese and Ayurvedic medicine.[3] It is, however, less well documented but still widely used in the traditional medicine of the tribes of Papua New Guinea. With only 99000 species of the estimated 3.5 million species of fungi existent on the planet described, the use of traditional knowledge in guiding us toward new bioactive compounds with demonstrated efficacy, and the inherent low toxicity provides a significant advantage over random screening.[4] The biodiversity of Papua New Guinea and its highly challenging terrain, limiting contact, have coupled to make this country one of the remaining frontiers in ethnomycology.

Boletopsin 7 (**1**) was first isolated by Nozoe and co-workers in 1992 from the Japanese mushroom *Boletopsis leucomelas* and was found to possess 5-lipoxygenase inhibitory activity.[5] Subsequently, in 2013 we reported the isolation of boletopsin 7, along with the two new compounds boletopsins 11 (**2**) and 12 (**3**), from a *Boletopsis* sp. of mushroom collected from the Lufa district in the Eastern Highlands Province in Papua New Guinea.[6] The edible mushroom is commonly used by the Kiovi tribe as a treatment for gastrointestinal complaints. It was shown that **1**−**3** were active against a panel of human pathogenic bacteria. The boletopsins are highly oxygenated *p*-terphenyl compounds containing a dibenzofuran moiety, with the fully functionalized central aromatic ring presenting a synthetic challenge. We report here the first syntheses of boletopsins 7, 11, and 12 (**1**−**3**).

RESULTS AND DISCUSSION

There are a number of syntheses of *p*-terphenyl compounds described in the literature.[7] We required a synthetic pathway that would allow for the preparation of the dibenzofuran moiety as well as the selective methylation of the 4-, 7-, and 8-hydroxy entities. Examining boletopsin 11 (**2**) in a retrosynthetic manner, we envisaged that the diacetate could be afforded by the deprotection and acetylation of methylenedioxy-protected dibenzofuran **4**, which itself could be formed from the Ullmann type condensation of the appropriate biaryl chloroformate (**5**), derived from a Baeyer−Villiger oxidation of benzaldehyde **6**. The *p*-terphenyl carbon skeleton could be prepared rapidly from sequential Suzuki−Miyaura couplings of sesamol derivative **8** and phenylboronic acids **7** and **9** (Scheme 1).

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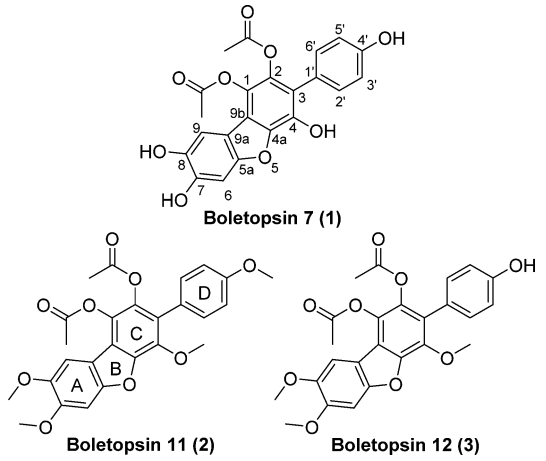
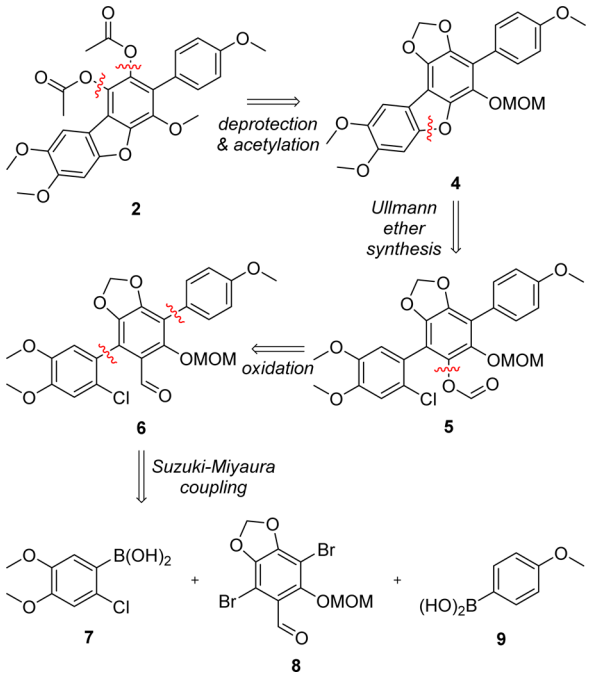


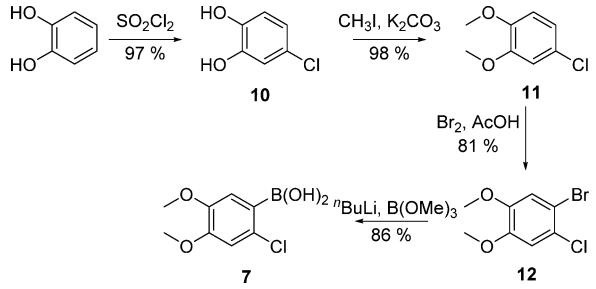
Scheme 1. Retrosynthetic Analysis of Boletopsin 11 (2)



We proposed that boletopsin 7 (**1**) could come from global demethylation of boletopsin 11 (**2**), and it was reasoned that preparation of boletopsin 12 (**3**) could be achieved by the stoichiometric addition of a methylating agent (Scheme 2). It was hypothesized that the 4′-hydroxyl would be the least acidic due to the lack of an adjacent hydrogen-bonding moiety.

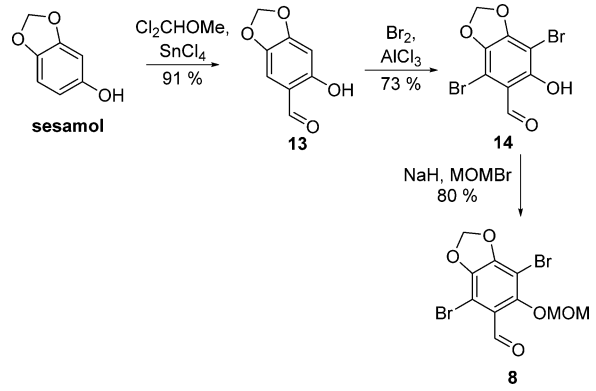
In this manner work began with the preparation of phenylboronic acid **7**. Chlorination of commercially available catechol was achieved with sulphuryl chloride in excellent yield (97%) on a gram scale (12.7 g) to give **10**. Methylation was then performed using methyl iodide and potassium carbonate to give the dimethoxybenzene **11**, which was brominated with molecular bromine to afford the phenyl bromide **12** in excellent yield (79% from **10**). Formation of the boronic acid was achieved by treatment of **12** with *n*-butyllithium followed by trimethyl borate and a subsequent acidic workup to give boronic acid **7** (86%) (Scheme 3), while 4-methoxyphenylboronic acid (**9**) was prepared from 4-bromoanisole via an analogous procedure.[8]

Scheme 3. Synthesis of Boronic Acid 7

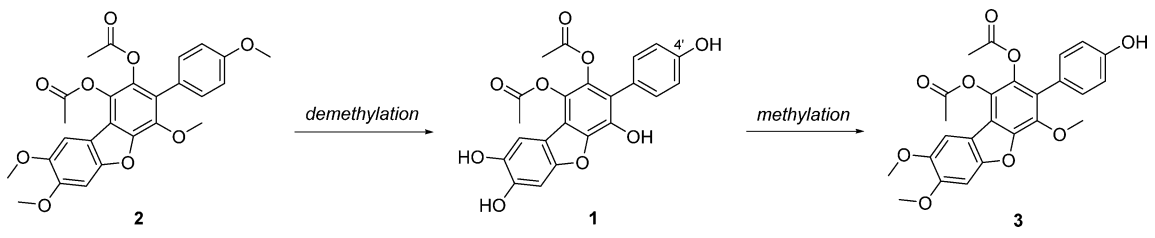


The C ring of the boletopsins was prepared by initially formylating commercially available sesamol using Rieche formylation conditions.[9] Subsequent dibromination of benzaldehyde **13** was achieved with molecular bromine and aluminum chloride employed as a strong Lewis acid, to give dibromide **14**. Without the addition of a strong Lewis acid only monobromination was observed. Subsequent protection with methoxymethyl bromide gave benzaldehyde **8** in good yield (53% from sesamol). Optimization revealed that the methoxymethylation proceeded in greater yields if the reaction temperature was not allowed to rise above 0 °C (Scheme 4).

Scheme 4. Synthesis of the C Ring Precursor, Dibromide 8



Scheme 2. Proposed Synthetic Route to Boletopsins 7 (1) and 12 (3)



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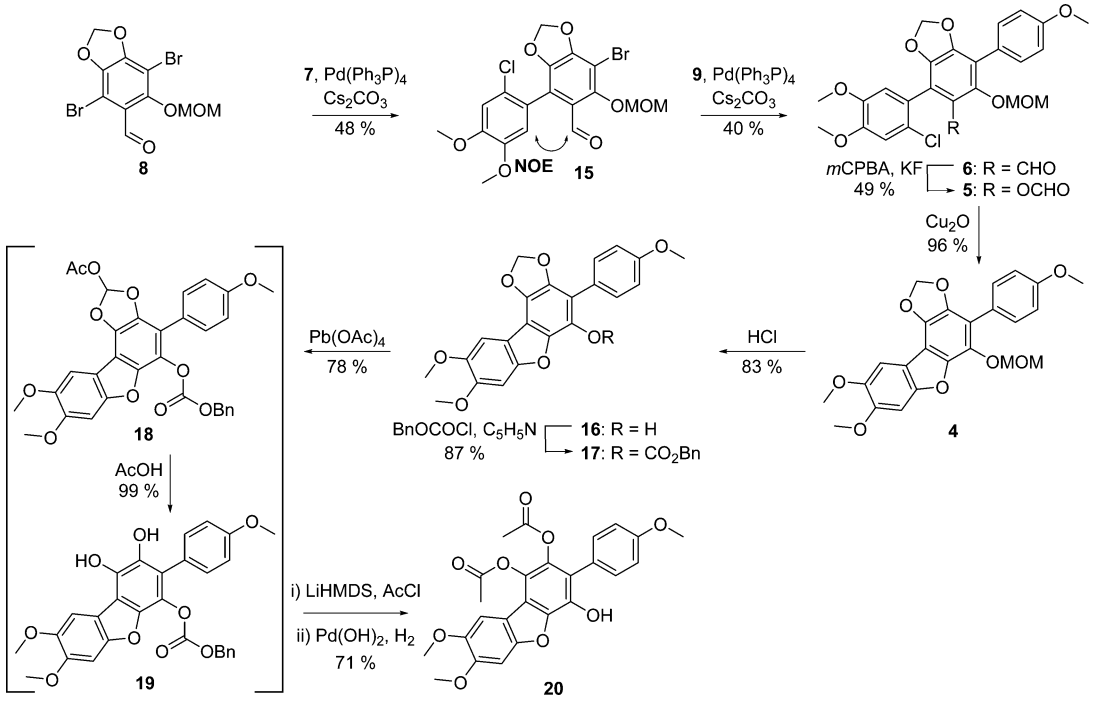
Scheme 5. Synthesis of *p*-Terphenyl Dibenzofuran 20



With dibromide **8** and phenylboronic acids **7** and **9** in hand, the Suzuki–Miyaura couplings could be investigated. At this stage we became aware of the work by Takahashi and co-workers, detailing their synthesis of vialinin B, which indicated that coupling ortho to the formyl moiety of **8** would occur first.[10] Despite this precedent, initial attempts at the coupling of chlorophenylboronic acid **7** and dibromide **8** proved ineffective, with this particular coupling proving very sensitive to the presence of oxygen. Without careful degassing via a freeze–pump–thaw process the reaction would not proceed at all, but with the degassing the reaction returned an acceptable yield of the biphenyl **15** (48%). Selective irradiation of the aldehydic proton resonance at 9.96 ppm showed a NOE to the aromatic singlet at 6.73 ppm, confirming the regioselectivity of coupling suggested by the work of Takahashi. Diagnostically, the methylene protons of the methoxymethyl ether and methylenedioxy protecting groups both appeared as discrete AB spin systems. Using the optimized conditions for the Suzuki–Miyaura reaction, **15** was coupled with *p*-methoxyphenylboronic acid (**9**) in acceptable yield to give *p*-terphenyl **6** (Scheme 5).

Formation of the dibenzofuran moiety was envisaged to proceed via an Ullmann ether synthesis; however, the initial step involving Baeyer–Villiger oxidation of benzaldehyde **6** to the corresponding formate **5** proved troublesome. Addition of *m*-chloroperbenzoic acid at a variety of temperatures yielded either no reaction or decomposition. Exploration of other oxidizing conditions such as the use of diphenyl selenide and hydrogen peroxide, reported to be a milder process, also resulted in decomposition.[11] The use of conditions proposed by Camps and co-workers using *m*-chloroperbenzoic acid with the addition of a large excess of potassium fluoride at 0 °C for 1 h resulted in a moderate yield (49%) of the desired formate **5**,[12] with the potassium fluoride acting to both activate the *m*-chloroperbenzoic acid and precipitate the resulting *m*-chlorobenzoic acid byproduct. It was found that the phenol resulting from hydrolysis of formate **5** was unstable, and as such the Ullmann ether synthesis was performed directly with formate **5** in the presence of copper(I) oxide and pyridine to give dibenzofuran **4** in excellent yield (96%) (Scheme 5).

With the core structure in hand, it only remained for installation of the acetate groups. Attempted acetoxylation of the methylenedioxy unit in **4** with lead(IV) acetate resulted in decomposition. Takahashi and co-workers had previously shown that the electron-rich methoxymethyl ether protecting group affected the stability of the methylenedioxy moiety;[10] therefore, substitution of the methoxymethyl ether with a benzylcarbonate group was achieved by first deprotection using anhydrous hydrogen chloride solution to give **16** followed by treatment with benzyl chloroformate in pyridine to give benzyl carbonate **17**. Treatment of **17** with lead(IV) acetate resulted in the formation of the unstable acetate **18** in good yield (78%), although the half-life was determined by proton NMR spectroscopy to be approximately 1 h. As such, acetate **18** was immediately treated with acetic acid to give catechol **19**, which was also found to be highly unstable. Exposure of the crude catechol to lithium hexamethyldisilazide in THF at −78 °C followed by addition of acetyl chloride showed the formation of a new, albeit unstable, compound by thin-layer chromatography, which upon immediate treatment with Pearlman's catalyst under an atmosphere of hydrogen gratifyingly yielded the stable dibenzofuran **20** in good yield (71% from **19**) (Scheme 5). Despite the instability of the intermediates, rapid processing of the material resulted in a reproducible yield over the three steps in the transformation of **17** to **20** of greater than 50%.

Methylation of **20** with potassium carbonate and methyl iodide gave boletopsin 11 (**2**) in excellent yield (91%) (Scheme 6). The characteristic data for synthetic boletopsin 11 were identical with those of natural boletopsin 11.[6] The formation of boletopsin 7 (**1**) was achieved by the treatment of **20** with boron tribromide at −78 °C, giving the tetrahydroxy *p*-

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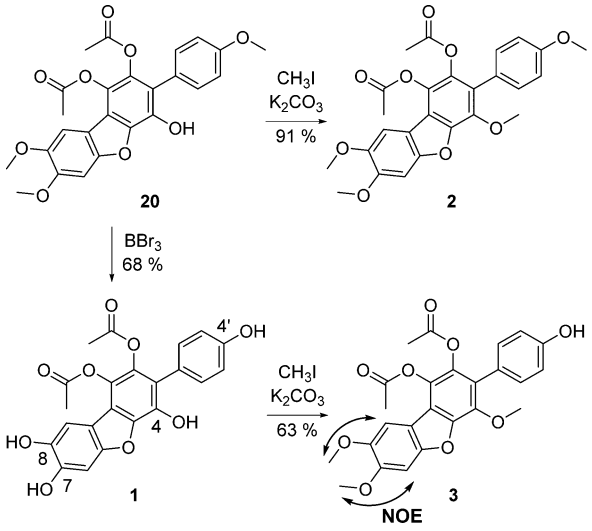
Scheme 6. Synthesis of Boletopsins 7 (1), 11 (2), and 12 (3)



terphenyl in 68% yield (Scheme 6). The data for synthetic boletopsin 7 were identical with those of the natural boletopsin 7.[5] Treatment of boletopsin 7 (**1**) with an excess of methyl iodide in the presence of potassium carbonate resulted in the formation of both boletopsin 11 (**2**, 29%) and a trimethoxy phenyldibenzofuran compound. Separation via HPLC yielded the desired boletopsin 12 (**3**) in good yield (63%). Confirmation of the methylation pattern was performed using 1D NOE experiments showing NOEs from 7-OMe to 6-H and from 8-OMe to 9-H (Scheme 6). The confirmation of methylation of the hydroxy at position 4 was given by the downfield shift of the carbon at 4-OMe (61.2 ppm) characteristic of methylation at this position. We propose that the regioselectivity is explained by the different acidities of 4-OH, 7-OH, and 8-OH versus 4′-OH in **1** due to the presence of a neighboring hydrogen-bonding moiety. The data for synthetic boletopsin 12 matched those of natural boletopsin 12.[6]

The synthetic boletopsins 7 (**1**), 11 (**2**), and 12 (**3**), as well as the two synthetic intermediates **16** and **20**, were tested against a panel of pathogenic bacteria in order to evaluate their antibiotic activity. The compounds were subjected to biological assays against clinical strains of *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Mycobacterium smegmatis*. Kanamycin was employed in the assay as a positive control and for comparison (Table 1).

An MIC value of 631 μg/mL was observed for boletopsin 11 (**2**) toward *P. aeruginosa*; all other compounds displayed MICs greater than 650 μg/mL. The purified synthetic compounds **1**–**3** showed activity comparable to that of the natural isolates,[6] while the activity of **16** and **20** suggested that the 4′-OMe group is not crucial in the activity of the boletopsins.

Table 1. Antibiotic Activity of 1–3, 16, and 20 showing 50% Inhibition of Selected Strains

	IC_{50} (μg/mL)					
strain[a]	kan[b]	**1**	**2**	**3**	**16**	**20**
S. epidermidis	90	251	213	475	420	582
E. coli	<1	>650	398	>650	515	592
P. aeruginosa	209	220	261	403	425	459
M. smegmatis	<1	432	89	352	304	384

[a]Clinical strains obtained from The Canberra Hospital. [b]Kanamycin.

CONCLUSIONS

An efficient route to the traditional medicinally relevant boletopsin class of compounds has been shown, culminating in the first syntheses of boletopsins 7, 11, and 12, achieved in 13, 13, and 14 steps, respectively, from commercially available sesamol. The key steps included sequential Suzuki–Miyaura couplings to rapidly build the *p*-terphenyl skeleton and an Ullmann ether synthesis utilizing a formate ester to prepare the dibenzofuran moiety. The synthetic natural products **1**–**3** as well as intermediates **16** and **20** were found to have weak antibiotic activity toward a panel of human pathogenic bacteria.

EXPERIMENTAL SECTION

General Experimental Conditions. Melting points are uncorrected. ^{1}H (300, 400, or 500 MHz) and ^{13}C NMR (75, 100, or 125 MHz) spectra were recorded at 298 K, at 300 MHz, 400 MHz, 500 and 75 MHz, 100 or 125 MHz, respectively. Chemical shifts are reported in ppm (δ).^{1}H NMR spectra are referenced to the resonance from residual $CHCl_3$ at 7.26 ppm and the central peak in the resonance from residual CHD_2COCD_3 at 2.05 ppm. ^{13}C NMR spectra are referenced to the central peak in the signal from $CDCl_3$ at 77.0 ppm and the central peak in the resonance from $(CD_3)_2CO$ at 29.8 ppm. The appearance and multiplicities of ^{1}H resonances are expressed by the abbreviations s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and combinations thereof for more highly coupled systems. ^{13}C NMR experiments were run as proton-decoupled spectra. Assignments were made according to literature precedent or using 2D NMR data including HSQC, HMBC, and NOESY data. MS (EI) and HRMS (EI) were recorded operating at 70 eV. Positive ionization was detected unless otherwise indicated. IR spectra were recorded as a neat solid or film. UV spectra were measured using a 1 cm solution cell in the solvent indicated. Spectral maxima are recorded in nm (log ε). Thin-layer chromatography (TLC) was run on silica gel 60 F_{254} aluminum backed plates and were run in the eluting system described for each plate. Plates were viewed under UV light (254 nm) and/or by development in potassium permanganate (100 mL of water, 1 g of $KMnO_4$, 6 g of K_2CO_3, 0.2 g of NaOH) or ceric phosphomolybdic acid dip (100 mL of water, 5 g of $12MoO_3 \cdot H_3PO_4$, 0.6 g of $Ce(SO_4)_2$, 6 mL of concentrated H_2SO_4). Flash chromatography was performed under pressure using silica gel (230–400 mesh) as solid support and HPLC-graded solvents as eluent. Semipreparative HPLC was performed using a 5 μm C18 250 × 10.00 mm column and a diode array detector for eluate detection. Elution was carried out using Milli-Q water and prefiltered (0.45 μm nylon membrane filter) methanol with detection at 243 nm. All solvents were dried and distilled either immediately prior to use or stored as appropriate. Ethereal solvents Et_2O and THF were refluxed over sodium and benzophenone. Toluene was refluxed over sodium, CH_2Cl_2 was refluxed with and distilled from CaH_2, and acetone was refluxed with and distilled from Na_2SO_4. The petroleum ether fraction used was 60–80 °C unless otherwise stated. Atoms are numbered according to the compound's IUPAC name.

4-Chlorobenzene-1,2-diol (10). To a solution of catechol (10.0 g, 90.8 mmol) in anhydrous ether (100 mL) at 0 °C was added sulfuryl chloride (16.1 mL, 27.0 g, 200 mmol) dropwise over 2 h. The solution was then warmed to room temperature and the solvent was left to evaporate under a stream of nitrogen, yielding the title compound as a white solid (97%, 12.7 g, 88.1 mmol). Mp: 89–91 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.86 (1H, m, 3-H), 6.77 (2H, m, 5,6-H), 5.35 (2H, broad s, 1,2-OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.2 (2-C), 142.2 (1-C), 120.8 (4-C), 116.0 (5-C), 115.7 (two coincident signals). IR (neat): ν_{max} 3416, 3328 cm^{-1}. MS (EI) *m*/*z*: 146 (25, $[M + 2]^{+\bullet}$), 144 (100, $[M]^{+\bullet}$). HRMS (EI) *m*/*z*: $[M]^{+\bullet}$ calcd for $C_6H_5O_2{}^{35}Cl$ 143.9978; found 143.9980.

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4-Chloro-1,2-dimethoxybenzene (11). **10** (12.7 g, 88.1 mmol) was dissolved in acetone (120 mL), and potassium carbonate (27.6 g, 200 mmol) was added. Methyl iodide (12.4 mL, 28.4 g, 200 mmol) was then added dropwise to the stirred suspension. The solution was heated to reflux and stirred for 16 h, at which time it was cooled and filtered and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and washed with water and brine. The organic layer was then separated, dried with $MgSO_4$, filtered, and concentrated to give a yellow oil. Purification was achieved by distillation at 3.0 mm/Hg at 120 °C to yield the title compound as a colorless oil (98%, 14.8 g, 86.3 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.88 (1H, dd, ^3J = 6.4, ^4J = 1.8 Hz, 5-H), 6.85 (1H, d, ^4J = 1.8 Hz, 3-H), 6.78 (1H, d, ^3J = 6.4 Hz, 6-H), 3.87 (3H, s, OCH_3), 3.86 (3H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 151.0 (2-C), 149.1 (1-C), 120.9 (4-C), 116.1, 116.1, 115.8, 55.7 (OCH_3), 55.4 (OCH_3). IR (neat): ν_{max} 2684, 1307 cm^{-1}. MS (EI) *m*/*z*: 174 (25, $[M + 2]^{+\bullet}$), 172 (100, $[M]^{+\bullet}$), 157 (50). HRMS (EI) *m*/*z*: $[M]^{+\bullet}$ calcd for $C_8H_9O_2{}^{35}Cl$ 172.0291; found 172.0291.

1-Bromo-2-chloro-4,5-dimethoxybenzene (12). To a solution of **11** (2.65 g, 15.4 mmol) in acetic acid (30 mL) was added bromine (0.790 mL, 2.45 g, 15.4 mmol). After the mixture was stirred for 3 h, saturated aqueous $Na_2S_2O_3$ was added and the solution was extracted with EtOAc. The organic layer was then washed with saturated $NaHCO_3$, dried with $MgSO_4$, filtered, and concentrated to give the title compound as a white solid (81%, 3.14 g, 12.4 mmol). Mp: 77−79 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.04 (1H, s, 6-H), 6.93 (1H, s, 3-H), 3.86 (6H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 148.9 (4-C), 148.3 (5-C), 125.6 (2-C), 115.8 (6-C), 112.9 (3-C), 112.2 (1-C), 56.3 (OCH_3), 56.3 (OCH_3). IR (neat): ν_{max} 2670, 1325 cm^{-1}. MS (EI) *m*/*z*: 254 (35, $[M + 4]^{+\bullet}$), 252 (98, $[M + 2]^{+\bullet}$), 250 (100, $[M]^{+\bullet}$), 236 (55), 128 (70). HRMS (EI) *m*/*z*: $[M]^{+\bullet}$ calcd for $C_8H_8O_2{}^{35}Cl{}^{79}Br$ 249.9396; found 249.9397.

(2-Chloro-4,5-dimethoxyphenyl)boronic Acid (7). A solution of **12** (3.14 g, 12.4 mmol) in THF (50 mL) was cooled to −78 °C, and *n*-butyllithium (1.10 M, 12.4 mL, 13.6 mmol) was added dropwise with stirring. After the mixture was stirred for 1 h, trimethylborate (1.53 mL, 1.42 g, 13.6 mmol) was added dropwise and the solution was slowly warmed to room temperature. Aqueous 0.5 M HCl was added, and the solution was stirred for a further 1 h, at which time it was extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$, water, and brine, dried with $MgSO_4$, filtered, and concentrated to yield a brown solid. Purification was achieved by trituration from 50% EtOAc in petroleum ether, providing the title compound as an amorphous white solid (86%, 2.31 g, 10.7 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (1H, s, 6-H), 6.83 (1H, s, 3-H), 3.91 (3H, s, OCH_3), 3.90 (3H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 151.8 (4-C), 147.6 (5-C), 131.4 (2-C), 118.3 (6-C), 112.4 (3-C), 56.1 (OCH_3), 56.0 (OCH_3). IR (neat): ν_{max} 3042, 2876 cm^{-1}. MS (EI) *m*/*z*: 218 (40, $[M + 2]^{+\bullet}$), 216 (40, $[M]^{+\bullet}$), 84 (100). HRMS (EI) *m*/*z*: $[M]^{+\bullet}$ calcd for $C_8H_{10}O_4{}^{11}B{}^{35}Cl$ 216.0361; found 216.0360.

6-Hydroxybenzo[*d*][1,3]dioxole-5-carbaldehyde (13). Sesamol (1.00 g, 7.25 mmol) was dissolved in CH_2Cl_2 (20 mL), tin(IV) chloride (1.02 mL, 2.27 g, 8.70 mmol) was added, and the solution was cooled to 0 °C. Dichloro(methoxy)methane (0.71 mL, 0.92 g, 8.00 mmol) was then added dropwise as the mixture was warmed to room temperature and stirred for 3 h. The mixture was poured over ice, and the water layer was separated and extracted once with CH_2Cl_2. The organic layer was washed with aqueous 1 M HCl and brine and was then passed through a small column packed with $MgSO_4$. The solvent was then removed in vacuo to yield the title compound as an off-white solid (91%, 1.09 g, 6.57 mmol). Mp: 124−127 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.79 (1H, s, 6-OH), 9.62 (1H, s, 5-CHO), 6.86 (1H, s, 4-H), 6.47 (1H, s, 7-H), 6.02 (2H, s, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.7 (CHO), 161.5 (7a-C), 155.2 (6-C), 141.3 (3a-C), 113.6 (5-C), 109.3 (4-C), 102.1 (2-C), 98.4 (7-C). IR (neat): ν_{max} 3419, 1618 cm^{-1}. MS (EI) *m*/*z*: 166 (100, $[M]^{+\bullet}$), 165 (90). HRMS (EI) *m*/*z*: $[M]^{+\bullet}$ calcd for $C_8H_6O_4$ 166.0266; found 166.0264.

4,7-Dibromo-6-hydroxybenzo[*d*][1,3]dioxole-5-carbaldehyde (14). To a solution of **13** (1.09 g, 6.57 mmol) in CH_2Cl_2 (60 mL) was added aluminum chloride (0.40 g, 3.00 mmol), and bromine (1.68 mL, 5.25 g, 32.9 mmol), and the solution was stirred at room temperature for 3 days. The reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and extracted with CH_2Cl_2. The organic layer was then washed with saturated aqueous $NaHCO_3$ and brine, dried with $MgSO_4$, filtered, and concentrated to give the title compound as a yellow solid (73%, 1.55 g, 4.78 mmol). Mp: 198−199 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.42 (1H, s, 6-OH), 10.00 (1H, s, 5-CHO), 6.17 (2H, s, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.4 (CHO), 160.3 (7a-C), 152.8 (6-C), 139.8 (3a-C), 110.8 (4-C), 102.7 (2-C), 101.6 (5-C), 90.5 (7-C). IR (neat): ν_{max} 3447, 1627 cm^{-1}. MS (EI) *m*/*z*: 326 (48, $[M + 4]^{+\bullet}$), 324 (100, $[M + 2]^{+\bullet}$), 322 (52, $[M]^{+\bullet}$). HRMS (EI) *m*/*z*: $[M]^{+\bullet}$ calcd for $C_8H_4O_4{}^{79}Br_2$ 321.8476; found 321.8474.

4,7-Dibromo-6-(methoxymethoxy)benzo[*d*][1,3]dioxole-5-carbaldehyde (8). Sodium hydride (60% oil dispersion, 0.25 g, 6.20 mmol) was washed with petroleum spirits and then added to a solution of **8** (1.00 g, 3.10 mmol) in DMF (10 mL) at 0 °C, and the solution was stirred for 30 min. Bromomethyl methyl ether (0.38 mL, 0.58 g, 4.65 mmol) was then added dropwise, and the solution was stirred at 0 °C for 1.5 h. While still at 0 °C aqueous NH_4Cl was added and the resulting solution was extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, and concentrated to give a brown oil. Purification was achieved via flash column chromatography on silica using 66% EtOAc in petroleum ether as eluent (R_f = 0.4), yielding the title compound as a white solid (80%, 0.913 g, 2.48 mmol). Mp: 138−140 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.17 (1H, s, 5-CHO), 6.20 (2H, s, 2-H), 5.12 (2H, s, OCH_2OCH_3), 3.62 (3H, s, OCH_2OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 188.27 (CHO), 155.0 (7a-C), 150.3 (6-C), 144.0 (3a-C), 122.24 (4-C), 102.7 (2-C), 102.0 (OCH_2OCH_3), 100.4 (5-C), 90.5 (7-C), 58.51 (OCH_2OCH_3). IR (neat): ν_{max} 1632 cm^{-1}. MS (EI) *m*/*z*: 372 (48, $[M + 2]^{+\bullet}$), 370 (100, $[M + 2]^{+\bullet}$), 368 (52, $[M]^{+\bullet}$). HRMS (EI) *m*/*z*: $[M]^{+\bullet}$ calcd for $C_{10}H_8O_5{}^{79}Br_2$ 367.8738; found 367.8739.

7-Bromo-4-(2-chloro-4,5-dimethoxyphenyl)-6-(methoxymethoxy)benzo[*d*][1,3]dioxole-5-carbaldehyde (15). A solution of **8** (319 mg, 0.867 mmol), **7** (207 mg, 0.954 mmol), cesium carbonate (565 mg, 1.73 mmol), water (0.30 mL) and toluene (15 mL) was degassed by a freeze/thaw process three times and placed under an atmosphere of argon to ensure O_2 was not present in the reaction vessel. Tetrakis(triphenylphosphine)palladium (50.0 mg, 45.0 μmol) was then added, and the solution was stirred at reflux for 16 h. The reaction was quenched by the addition of water, and the solution was extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, and concentrated to give a brown solid. Purification was achieved via flash column chromatography on silica using 25% EtOAc in petroleum ether as eluent (R_f = 0.2), yielding a white amorphous solid (48%, 190 mg, 0.413 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.96 (1H, s, 5-CHO), 6.95 (1H, s, 3′-H), 6.73 (1H, s, 6′-H), 6.10 (2H, m, 2-CH_AH_B), 5.18 (1H, d, ^2J = 4.7 Hz, $OCH_AH_BOCH_3$), 5.16 (1H, d, ^2J = 4.7 Hz, $OCH_AH_BOCH_3$), 3.90 (3H, s, 4′- or 5′-$_3$), 3.83 (3H, s, 4′- or 5′-OCH_3), 3.64 (3H, s, $OCH_AH_BOCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 188.5 (5-CHO), 154.0 (7a-C), 150.7 (6-C), 149.6 (4′-C), 147.7 (5′-C), 142.8 (3a-C), 124.5 (1′-C), 123.0, 122.9, 120.0, 113.1 (3′-C), 112.3 (6′-C), 102.6 (7-C), 101.8 (2-C), 97.7 (OCH_2OCH_3), 58.4 (OCH_3), 56.1 (two coincident signals). IR (neat): ν_{max} 2968, 1692, 1600 cm^{-1}. MS (ESI) *m*/*z*: 485 (40, $[M + 4 + Na]^+$), 483 (96, $[M + 2 + Na]^+$), 481 (100, $[M + Na]^+$). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{18}H_{16}O_7{}^{35}Cl{}^{79}Br{}^{23}Na$ 480.9666; found 480.9669.

4-(2-Chloro-4,5-dimethoxyphenyl)-6-(methoxymethoxy)-7-(4-methoxyphenyl)benzo[*d*][1,3]dioxole-5-carbaldehyde (6). A solution of **15** (190 mg, 0.413 mmol), **9** (69 mg, 0.454 mmol), cesium carbonate (269 mg, 826 mmol), water (0.20 mL), and toluene (10 mL) was degassed by a freeze/thaw process three times and placed under an atmosphere of argon. Tetrakis(triphenylphosphine)palladium (50.0 mg, 45.0 μmol) was then added, and the solution was stirred at reflux for 16 h. The reaction was quenched by the addition of water, and the solution was extracted with EtOAc. The organic layer was dried with $MgSO_4$, filtered, and concentrated to give a brown solid. Purification was achieved via flash column chromatography on silica using 50% EtOAc in petroleum ether as eluent (R_f = 0.4), yielding a white amorphous solid (40%, 78.0 mg, 0.160 mmol). ^{1}H NMR (300

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MHz, $CDCl_3$): δ 10.09 (1H, s, 5-CHO), 7.56 (2H, m, 2″,6″-H), 7.00 (2H, m, 3″,5″-H), 6.98 (1H, s, 3′-H), 6.80 (1H, s, 6′-H), 6.03 (2H, m, 2-H_AH_B), 4.75 (2H, s, OCH_2OCH_3), 3.91 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.15 (3H, s, OCH_2OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.5 (CHO), 159.3 (4″-C), 154.5 (7a-C), 149.7 (6-C), 149.3 (4′-C), 147.6 (5′-C), 142.6 (3a-C), 131.3 (2″,6″-C), 131.1 (1′-C), 124.6 (2′-C), 123.8 (1″-C), 123.2, 122.3, 113.8 (3″,5″-C), 113.6, 113.3 (3′-C), 112.3 (6′-C), 102.1 (2-C), 101.1 (OCH_2OCH_3), 57.7 (OCH_2OCH_3), 56.1 (OCH_3), 56.0 (OCH_3), 55.2 (OCH_3). IR (neat): ν_{max} 3034, 1687, 1598 cm^{-1}. MS (ESI) *m/z*: 511 (40, $[M + 2+Na]^+$), 509 (100, $[M + Na]^+$). HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{25}H_{24}O_8{}^{35}Cl$ 487.1160; found 487.1155.

4-(2-Chloro-4,5-dimethoxyphenyl)-6-(methoxymethoxy)-7-(4-methoxyphenyl)benzo[*d*][1,3]dioxol-5-yl Formate (5). 6 (78.0 mg, 0.160 mmol) was added to a solution of potassium fluoride (46 mg, 0.800 mmol) and *m*-CPBA (50%, 55 mg, 0.160 mmol) in CH_2Cl_2 (20 mL) at 0 °C. After it was stirred for 2 h the reaction mixture was filtered and the solvent removed to give a yellow oil. Purification was performed by flash column chromatography on silica using 50% EtOAc in petroleum ether as eluent (R_f = 0.4), yielding the title compound as a colorless amorphous solid (49%, 40.0 mg, 0.0796 mmol). ^1H NMR (300 MHz, $CDCl_3$): δ 8.08 (1H, s, OCHO), 7.56 (2H, m, 2″,6″-H), 6.98 (2H, m, 3″,5″-H), 6.95 (1H, s, 3′-C), 6.78 (1H, s, 6′-C), 5.95 (2H, s, 2-H), 4.62 (2H, s, OCH_2OCH_3), 3.87 (3H, s, OCH_3), 3.82 (6H, s, OCH_3), 3.14 (3H, s, OCH_2OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.6 (OCHO), 159.3 (4″-C), 149.7 (4′-C), 147.6 (5′-C), 143.1 (6-C), 142.0 (7a-C), 140.7 (3a-C), 135.7, 131.2 (2″,6″-C), 131.2 (1′-C), 125.3 (2′-C), 123.6 (1″-C), 121.4, 118.2, 113.7 (3″,5″-C), 112.4, 109.9, 101.7 (2-C), 99.2 (OCH_2OCH_3), 57.3 (OCH_2OCH_3), 56.1 (OCH_3), 56.0 (OCH_3), 55.2 (OCH_3). IR (neat): ν_{max} 3069, 1752, 1610 cm^{-1}. MS (ESI) *m/z*: 527 (40, $[M + 2 + Na]^+$), 525 (100, $[M + Na]^+$), 483 (50). HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{25}H_{23}O_9{}^{35}Cl^{23}Na$ 525.0928; found 525.0915.

8,9-Dimethoxy-5-(methoxymethoxy)-4-(4-methoxyphenyl)-benzo[*b*][1,3]dioxolo[4,5-*e*]benzofuran (4). A solution of **5** (75 mg, 0.149 mmol) and copper(I) oxide (108 mg, 0.750 mmol) in anhydrous pyridine (5 mL) was degassed via a freeze/thaw process three times. The reaction mixture was then heated to relux for 16 h with stirring. After the mixture was cooled, EtOAc was added and the solution was then washed five times with water and then brine. The organic layer was dried with $MgSO_4$, filtered, and concentrated to yield the title compound as a brown amorphous solid (96%, 63.0 mg, 0.144 mmol). ^1H NMR (300 MHz, $CDCl_3$): δ 7.61 (2H, m, 2′,6′-H), 7.34 (1H, s, 7-H), 7.00 (2H, m, 3′,5′-H), 7.11 (1H, s, 10-H), 6.10 (2H, s, 2-H), 5.04 (2H, s, OCH_2OCH_3), 3.99 (3H, s, OCH_3), 3.98 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.21 (3H, s, OCH_2OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.0 (4′-C), 151.5, 149.8, 146.2 (6a-C), 145.0 (3a-C), 140.7 (5a-C), 135.1 (5-C), 132.4 (10c-C), 131.5 (2′, 6′-C), 124.7 (1′-C), 115.5 (10b-C), 113.6 (3′, 5′-C), 113.3 (4-C), 109.0 (10a-C), 103.5 (10-C), 101.8 (2-C), 98.5 (OCH_2OCH_3), 95.6 (7-C), 57.0 (OCH_2OCH_3), 56.5 (OCH_3), 56.2 (OCH_3), 55.2 (OCH_3). IR (neat): ν_{max} 3036, 1609 cm^{-1}. MS (EI) *m/z*: 438 (10, $[M]^{+\bullet}$), 407 (25), 269 (75), 200 (100). HRMS (EI) *m/z*: $[M]^{+\bullet}$ calcd for $C_{24}H_{22}O_8$ 438.1315; found 438.1313.

8,9-Dimethoxy-4-(4-methoxyphenyl)benzo[*b*][1,3]dioxolo-[4,5-*e*]benzofuran-5-ol (16). To a solution of **4** (60 mg, 0.137 mmol) in THF (5 mL) was added HCl (4.0 M in dioxane, 0.82 mL, 0.205 mmol), and the solution was stirred at room temperature for 2 h. After addition of water the solution was extracted with EtOAc and the organic layer was washed with water and brine, dried with $MgSO_4$, filtered, and concentrated to give a colorless amorphous solid. Purification was achieved via flash column chromatography on silica using 50% EtOAc in petroleum ether as eluent (R_f = 0.4), yielding the title compound as a colorless amorphous solid (83%, 50.0 mg, 0.114 mmol). ^1H NMR (300 MHz, $CDCl_3$): δ 7.56 (2H, m, 2′,6′-H), 7.32 (1H, s, 7-H), 7.06 (2H, m, 3′,5′-H), 7.02 (1H, s, 10-H), 6.06 (2H, s, 2-H), 5.26 (broad s, 1H, 5-OH), 3.98 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.85 (3H, s, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.0 (4′-C), 151.6, 149.8, 146.2 (6a-C), 145.0 (3a-C), 140.8 (5a-C), 135.1 (5-C), 132.5 (10c-C), 131.6 (2′,6′-C), 124.7 (1′-C), 115.5 (10b-C), 113.6 (3′,5′-C), 113.3 (4-C), 109.1 (10a-C), 103.6 (10-C), 101.8 (2-C), 95.6 (7-C), 56.5 (OCH_3), 56.3 (OCH_3), 55.3 (OCH_3). IR (neat): ν_{max} 3539, 3071, 1609 cm^{-1}. MS (EI) *m/z*: 394 (100, $[M]^{+\bullet}$), 351 (30). HRMS (EI) *m/z*: $[M]^{+\bullet}$ calcd for $C_{22}H_{18}O_7$ 394.1053; found *m/z* 394.1056.

Benzyl (8,9-Dimethoxy-4-(4-methoxyphenyl)benzo[*b*][1,3]-dioxolo[4,5-*e*]benzofuran-5-yl)carbonate (17). 16 (16 mg, 37.0 μmol) was dissolved in anhydrous pyridine (1 mL) and CH_2Cl_2 (0.5 mL) and cooled to 0 °C. Benzyl chloroformate (10 μL, 12 mg, 73.0 μmol) was added dropwise, and the solution was stirred for 30 min. The reaction was then quenched by the addition of water, and the solution was extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$, water, and brine, dried with $MgSO_4$, filtered, and concentrated to give a yellow amorphous solid. Purification was performed using flash column chromatography on silica using 30% EtOAc in petroleum ether as eluent (R_f = 0.2), yielding a colorless amorphous solid (87%, 17.0 mg, 32.2 μmol). ^1H NMR (300 MHz, $CDCl_3$): δ 7.47 (2H, m, 2″,6″-C), 7.40−7.25 (5H, m, 2−6-H), 7.33 (1H, s, 7′-H), 7.09 (1H, s, 10′-H), 6.91 (2H, m, 3″,5″-H), 6.14 (2H, s, 2′-H), 5.20 (2H, s, OCH_2Ph), 3.99 (OCH_3), 3.97 (OCH_3), 3.84 (OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3 (4″-C), 153.2 (CO_3), 151.8 (8′-C), 150.0 (9′-C), 146.4, 144.3, 140.9 (3′a-C), 140.6 (10′c-C), 137.0, 134.8, 131.0 (2″,6″-C), 128.6, 128.5 (3,5-C), 128.2 (2,6-C), 127.7, 123.2, 115.1, 113.9 (3″,5″-C), 113.1, 109.1 (10′b-C), 103.5 (10′-C), 102.1 (2′-C), 95.8 (7′-C), 70.5 (OCH_2Ph), 56.5 (OCH_3), 56.3 (OCH_3), 55.2 (OCH_3). IR (neat): ν_{max} 3030, 1764, 1609 cm^{-1}. MS (EI) *m/z*: 528 (20, $[M]^{+\bullet}$), 394 (90), 393 (100), 363 (50). HRMS (EI) *m/z*: $[M]^{+\bullet}$ calcd for $C_{30}H_{24}O_9$ 528.1420; found 528.1417.

5-(((Benzyloxy)carbonyl)oxy)-8,9-dimethoxy-4-(4-methoxyphenyl)benzo[*b*][1,3]dioxolo[4,5-*e*]benzofuran-2-yl Acetate (18). A solution of **17** (12 mg, 22.7 μmol) and lead tetraacetate (23 mg, 51.8 μmol) in toluene (3 mL) was heated to reflux for 2 h until the consumption of starting material was observed by TLC. After it was cooled, the reaction mixture was filtered through Celite and concentrated in vacuo, yielding the title compound as a colorless amorphous solid (78%, 10.4 mg, 17.7 μmol). The title compound was found to be unstable and was used directly in the next step without further purification. ^1H NMR (300 MHz, $CDCl_3$): δ 7.89 (1H, s, 2-H), 7.57 (2H, m, 2″,6″-H), 7.40−7.25 (6H, m, 2′-6′-H, 7-H), 7.10 (1H, s, 10-H), 6.92 (2H, m, 3″,5″-H), 5.20 (2H, s, OCH_2Ph), 4.00 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 2.09 (3H, s, $C(O)CH_3$). IR (neat): ν_{max} 3042, 1769, 1610, 1520 cm^{-1}. MS (EI) *m/z*: 586 (40, $[M]^{+\bullet}$), 567 (100). HRMS (EI) *m/z*: $[M]^{+\bullet}$ calcd for $C_{32}H_{26}O_{11}$ 586.1475; found 586.1473.

Benzyl (1,2-Dihydroxy-7,8-dimethoxy-3-(4-methoxyphenyl)dibenzo[*b*,*d*]furan-4-yl)carbonate (19). A solution of **18** (10 mg, 17.7 μmol) in acetic acid (2 mL) was stirred at room temperature for 30 min, at which time the solvent was removed in vacuo, yielding the title compound as an unstable brown amorphous solid (99%, 9.0 mg, 18 μmol) that was used immediately in the following step. ^1H NMR (300 MHz, $CDCl_3$): δ 7.48 (2H, m, 2″,6″-H), 7.42 (1H, s, 6′-H), 7.40−7.25 (5H, m, 2−6-H), 7.14 (1H, s, 9′-H), 6.95 (2H, m, 3″,5″-H), 5.22 (2H, s, OCH_2Ph), 4.03 (3H, s, OCH_3), 4.00 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 2.82 (1H, s, OH), 2.80 (1H, s, OH).

4-Hydroxy-7,8-dimethoxy-3-(4-methoxyphenyl)dibenzo-[*b*,*d*]furan-1,2-diyl Diacetate (20). A solution of **19** (30 mg, 58.1 μmol) in THF (5 mL) was cooled to −78 °C. Lithium hexamethyldisilazide (1.0 M, 128 μL, 128 μmol) was then added dropwise with stirring. After 30 min acetyl chloride (10 μL, 11 mg, 145 μmol) was added dropwise and the solution was warmed to room temperature. The solution was then concentrated in vacuo and the residue dissolved in EtOAc (5 mL). Palladium hydroxide on carbon (10 mg) was added and the solution was stirred vigorously under an atmosphere of hydrogen for 16 h. The reaction was then filtered and concentrated to give a brown solid. Purification was achieved via flash column chromatography on silica using 50% EtOAc in petroleum ether as eluent (R_f = 0.2), yielding the title compound as a stable brown amorphous solid (71%, 19.2 mg, 41.3 μmol). ^1H NMR (400

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MHz, acetone-d_6): δ 7.36 (1H, s, 6-H), 7.27 (1H, s, 9-H), 7.25 (2H, m, 2′,6′-H), 7.00 (2H, m, 3′,5′-H), 3.96 (3H, s, 7-OCH_3), 3.94 (3H, s, 8-OCH_3), 3.92 (3H, s, 4′-$OCHH_3$), 2.49 (3H, s, 1-OAc), 2.01 (3H, s, 2-OAc). ^{13}C NMR (125 MHz, acetone-d_6): δ 169.2 (2-CH_3CO), 169.0 (1-CH_3CO), 160.5 (4′-C), 152.5 (7-C), 148.3 (8-C), 146.9 (5a-C), 141.7 (4-C), 137.7 (4a-C), 132.6 (2′,6′-C), 132.6 (2-C), 126.8 (1-C), 126.2 (1′-C), 122.7 (3-C), 120.5 (9b-C), 114.6 (3′,5′-C), 114.5 (9a-C), 105.0 (9-C), 97.2 (6-C), 57.2 (8-OCH_3), 56.9 (7-OCH_3), 55.9 (4′-OCH_3), 20.8 (1-CH_3CO), 20.5 (2-CH_3CO). IR (neat): ν_{max} 3395, 1743, 1731, 1607 cm^{-1}. MS (EI) m/z: 466 (42, $[M]^{+\bullet}$), 407 (100). HRMS (EI) m/z: $[M]^{+\bullet}$ calcd for $C_{25}H_{22}O_9$ 466.1264; found 466.1265.

Boletopsin 11: 4,7,8-Trimethoxy-3-(4-methoxyphenyl)-dibenzo[*b*,*d*]furan-1,2-diyl Diacetate (2). Potassium carbonate (6 mg, 43 μmol) and iodomethane (3 μL, 7 mg, 50 μmol) were added to a solution of **20** (10 mg, 21.5 μmol) in acetone (5 mL) and stirred at reflux for 16 h. After it was cooled, the mixture was filtered and the solution was then concentrated to give a brown amorphous solid. Purification was performed by flash column chromatography on silica using 33% EtOAc in petroleum ether as eluent (R_f = 0.3), yielding the title compound as a brown amorphous solid (91%, 9.4 mg, 20 μmol). Purity was confirmed via HPLC using 40% methanol in water as eluent with a flow rate of 1.5 mL/min, resulting in a retention time of 24.08 min. ^{1}H NMR (400 MHz, acetone-d_6): δ 7.37 (1H, s, 6-H), 7.28 (1H, s, 9-H), 7.27 (2H, m, 2′,6′-H), 7.01 (2H, m, 3′,5′-H), 3.97 (3H, s, 7-OCH_3), 3.95 (3H, s, 4-OCH_3), 3.93 (3H, s, 8-OCH_3), 3.85 (3H, s, 4′-OCH_3), 2.49 (3H, s, 1-OAc), 2.01 (3H, s, 2-OAc). ^{13}C NMR (125 MHz, acetone-d_6): δ 168.7 (2-CH_3CO), 168.5 (1-CH_3CO), 160.0 (4′-C), 152.3 (7-C), 147.8 (8-C), 146.4 (5a-C), 141.2 (4-C), 137.2 (4a-C), 132.1 (2′,6′-C), 132.1 (2-C), 126.3 (1-C), 125.7 (1′-C), 122.1 (3-C), 120.0 (9b-C), 114.1 (3′,5′-C), 114.0 (9a-C), 104.5 (9-C), 96.7 (6-C), 61.1 (4-OCH_3), 56.6 (8-OCH_3), 56.4 (7-OCH_3), 55.4 (4′-OCH_3), 20.2 (1-CH_3CO), 20.0 (2-CH_3CO). IR (neat): ν_{max} 1756, 1741, 1602 cm^{-1}. MS (EI) m/z: 480 (25, $[M]^{+\bullet}$), 421 (100), 363 (55). HRMS (EI) m/z: $[M]^{+\bullet}$ calcd for $C_{26}H_{24}O_9$ 480.1420; found 480.1430. UV (CH_3OH) λ_{max} (log ε): 309 (3.62), 266 (3.75), 237 (4.19) nm.

Boletopsin 7: 4,7,8-Trihydroxy-3-(4-hydroxyphenyl)-dibenzo[*b*,*d*]furan-1,2-diyl Diacetate (1). A solution of **20** (10.0 mg, 21.5 μmol) in toluene (5 mL) was cooled to −78 °C, and boron tribromide (1.0 M, 100 μL, 100 μmol) was added dropwise. The solution was slowly warmed to room temperature and stirred for 5 h, at which time ice was added. The solution was then extracted with EtOAc, and the organic layer was dried with $MgSO_4$, filtered, and concentrated to give a brown amorphous solid. Purification was achieved via HPLC using 40% MeOH in water as eluent with a flow rate of 1.5 mL/min (R_t = 8.19 min), yielding a brown amorphous solid (68%, 6.2 mg, 15 μmol). ^{1}H NMR (400 MHz, acetone-d_6): δ 7.24 (1H, s, 9-H), 7.19 (2H, m, 2′,6′-H), 7.10 (1H, s, 6-H), 6.90 (2H, m, 3′,5′-H), 2.44 (3H, s, 1-OAc), 2.00 (3H, s, 2-OAc). ^{13}C NMR (125 MHz, acetone-d_6): δ 168.8 (2-CH_3CO), 168.5 (1-CH_3CO), 157.7 (4′-C), 151.7 (7a-C), 147.4 (8-C), 143.4 (4-C), 143.1 (5a-C), 138.3 (4a-C), 137.1 (2-C), 132.5 (2′,6′-C), 129.6 (1-C), 124.4 (1′-C), 121.6 (3-C), 119.0 (9b-C), 115.7 (3′,5′-C), 114.7 (9a-C), 107.2 (9-C), 99.0 (6-C), 20.2 (1-CH_3CO), 20.0 (2-CH_3CO). IR (neat): ν_{max} 3425, 1759, 1616 cm^{-1}. MS (EI) m/z: 424 (60, $[M]^{+\bullet}$), 382 (100), 340 (55). HRMS (EI) m/z: $[M]^{+\bullet}$ calcd for $C_{22}H_{16}O_9$ 424.0794; found 424.0791. UV (CH_3OH) λ_{max} (log ε): 380 (3.54), 297 (3.86), 258 (3.93), 210 (4.17) nm.

Boletopsin 12: 3-(4-Hydroxyphenyl)-4,7,8-trimethoxydibenzo[*b*,*d*]furan-1,2-diyl Diacetate (3). To a solution of **1** (10.0 mg, 23.6 μmol) in acetone (2 mL) were added potassium carbonate (19.6 mg, 142 μmol) and iodomethane (8.81 μL, 20.1 mg, 142 μmol), and the solution was stirred at reflux for 16 h. After it was cooled to room temperature, the solution was filtered and concentrated in vacuo to yield a brown amorphous solid. Purification was achieved via HPLC using 40% MeOH in water as eluent with a flow rate of 1.5 mL/min (R_t = 12.92 min) to give the title compound as an amorphous brown solid (63%, 15 μmol, 6.9 mg), as well as **2** (R_t = 8.11 min) as an amorphous brown solid (29%, 7.8 μmol, 2.9 mg). ^{1}H NMR (400 MHz, acetone-d_6): δ 7.36 (1H, s, 6-H), 7.28 (1H, s, 9-H), 7.18 (2H, m, 2′,6′-H), 6.92 (2H, m, 3′,5′-H), 3.97 (3H, s, 7-OCH_3), 3.94 (3H, s, 4-OCH_3), 3.92 (3H, s, 8-OCH_3), 2.49 (3H, s, 1-OAc), 2.02 (3H, s, 2-OAc). ^{13}C NMR (125 MHz, acetone-d_6): δ 168.9 (2-$COCH_3$), 168.6 (1-$COCH_3$), 157.9 (4′-C), 152.1 (7-C), 147.9 (8-C), 146.6 (5a-C), 141.3 (4-C), 137.3 (4a-C), 132.2 (2′,6′-C), 132.2 (2-C), 126.8 (1-C), 124.7 (1′-C), 120.0 (9b-C), 115.7 (3′,5′-C), 114.2 (9a-C), 104.6 (9-C), 96.8 (6-C), 61.2 (4-OCH_3), 56.8 (8-OCH_3), 56.5 (7-OCH_3), 20.4 (1-$COCH_3$), 20.1 (2-$COCH_3$). IR (neat): ν_{max} 3402, 1735, 1720, 1601 cm^{-1}. MS (EI) m/z: 466 (25, $[M]^{+\bullet}$), 407 (100). HRMS (EI) m/z: $[M]^{+\bullet}$ calcd for $C_{25}H_{22}O_9$ 466.1264; found 466.1266. UV (CH_3OH) λ_{max} (log ε): 292 (3.81), 247 (4.02), 208 (4.17) nm.

Determination of the Sensitivity of Selected Bacterial Strains against Selected Compounds. The sensitivity of four clinical bacterial strains (*Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Mycobacterium smegmatis*), were tested by a turbidity assay OD_{600} (optical density at λ 600 nm).[13] To prepare the inoculum, the bacterial suspension, adjusted to equal the density of 0.5 McFarland standard (OD_{600} = 0.08), was diluted 1:100 with Mueller Hinton broth (MHB) and 150 μL was used as an inoculum. A 40 μL portion of MHB was added to wells 1B−1H of sterile 96-well plates. An 80 μL portion of the unfiltered test sample (3 mg/mL) or appropriate antibiotic control (1 mg/mL) was dispensed into well 1A, and 40 μL was removed and serial 2-fold dilutions of the test sample were prepared directly on the plate. The plate was read at A_{600} to control for pre-existing turbidity of the samples before incubation. The plate was incubated at 37 °C for 18 h on a shaker incubator. After incubation, the plate was read at A_{600} to assess the relative turbidity (i.e., growth) of the treated cultures. IC_{50} (50% inhibition) was determined on the basis of a comparison with the average turbidity readings of the untreated control. The inhibition was calculated as

$$IC_{50} = 10^{[\log(A/B)\times(50-C)/(D-C)+\log B]}$$

where A is the higher concentration of the test compound of the two points on the graph that bracket 50% inhibition, B is the lower concentration of the test compound of the two points on the graph that bracket 50% inhibition, C is the inhibitory activity (%) at the concentration B, and D is the inhibitory activity (%) at the concentration A.

■ ASSOCIATED CONTENT

Supporting Information

Figures giving ^{1}H and ^{13}C NMR spectra for all synthetic compounds and analytical data for the synthesized natural products. This material is available free of charge via the Internet at http://pubs.acs.org.

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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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