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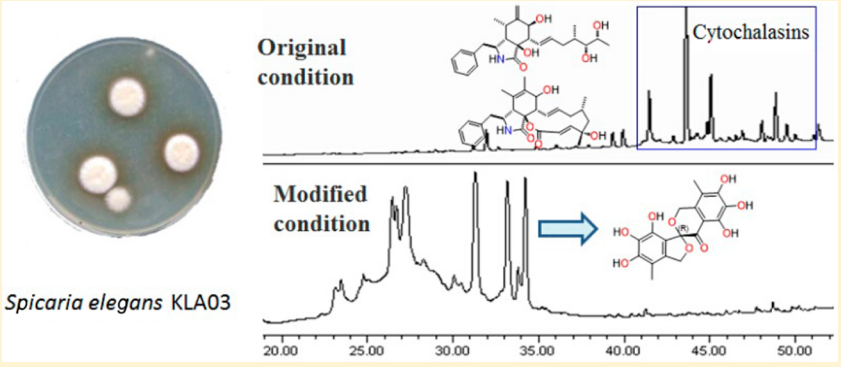
Eleganketal A, a Highly Oxygenated Dibenzospiroketal from the Marine-Derived Fungus *Spicaria elegans* KLA03

Yepeng Luan,[†] Hongjuan Wei,[†] Zhenpei Zhang,[†] Qian Che,[†] Yankai Liu,[†] Tianjiao Zhu,[†] Attila Mándi,[‡] Tibor Kurtán,[‡] Qianqun Gu,[†] and Dehai Li*[,†]

[†]Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China

[‡]Department of Organic Chemistry, University of Debrecen, POB 20, H-4010 Debrecen, Hungary

Supporting Information



ABSTRACT: Eleganketal A (**1**), a naturally occurring aromatic polyketide possessing a rare highly oxygenated spiro[isobenzofuran-1,3′-isochroman] ring system, was isolated from the fungus *Spicaria elegans* KLA03 by culturing it in a modified mannitol-based medium. The structure of **1** including the absolute configuration was determined by combining spectroscopic analysis, synthesis of the racemic permethylated analogue, chiral-phase HPLC separation, and TDDFT-ECD analysis.

Marine-derived fungi are a powerful resource to produce unique bioactive structures attracting the attention of chemists and biologists.[1] Although thousands of compounds with various chemical structures have been disclosed, most of the gene clusters encoding secondary metabolites are silent under a single culture condition.[2,3] To maximize the chemical diversity of fungal metabolites, the so-called "OSMAC" approach has proven to be a convenient and useful method by altering culture parameters, feeding precursors, adding enzyme inhibitors, etc.[3]

During our exploration for bioactive compounds from marine-derived fungi, the cytochalasins Z_9−Z_{15} (glucose-based medium under static conditions) have been isolated from the fungus *Spicaria elegans* KLA03, which derives from marine sediments collected in Jiaozhou Bay, China.[4] Guided by the OSMAC approach, 14 new polyketides of this family were further isolated including spicochalasin A and aspochalasins M−Q (starch and soybean-based medium upon shaking for 8 days),[5] 7-deoxycytochalasins Z_7 and Z_9 (adding a cytochrome P-450 inhibitor to glucose-based medium under static conditions),[6] aspochalasins R−T (starch and soybean-based medium, shaking for 14 days),[7] and cytochalasins Z_{21}−Z_{23} (adding D- and L-tryptophan to glucose-based medium, static conditions).[8] Attracted by the amazing ability of this strain to produce structurally diverse cytochalasin-type polyketides, more fermentation conditions were tested, and it was found that the HPLC-UV profile of the EtOAc extract changed dramatically when cultured in a modified mannitol-based medium (NH_4Cl as nitrogen source). From the broth, eleganketal A (**1**), possessing a rare substituted 3*H*-spiro-[isobenzofuran-1,3′-isochroman]-4′(1′*H*)-one skeleton, together with two biogenetically related known compounds, 4,5,6-trihydroxy-7-methyl-1,3-dihydroisobenzofuran (**2**)[9] and flavimycin A (**3**),[10] was isolated. The new structure of **1** including the absolute configuration was determined by a combination of spectroscopic analysis, chemical synthesis, chiral-phase HPLC analysis, and TDDFT-ECD calculations.

RESULTS AND DISCUSSION

The fermented whole broth (20 L) was obtained by culturing *S. elegans* KLA03 with shaking at 28 °C for 8 days in the modified mannitol medium. The EtOAc extract (20 g) was subjected to repeated silica gel and LH-20 column chromatography to yield the new compound **1** (8.0 mg).

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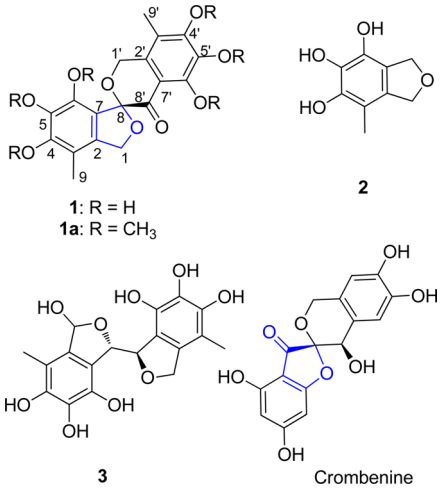


Eleganketal A (**1**), an amorphous, yellow powder, possessed the molecular formula $C_{18}H_{16}O_9$ according to the negative HRESIMS peak at *m*/*z* 375.0710. The 1D NMR data indicated two methyls, two methylenes, one ketal (δ_C 107.5, C-8), one ketone (δ_C 192.2, C-8′), and 14 quaternary carbons including 12 aromatic ones with six of them oxygenated, suggesting the presence of two hexasubstituted benzene rings.

The planar structure was tentatively proposed by analysis of the 2D NMR data (Figure 1). The 1,6-dioxaspiro[4.5]decane

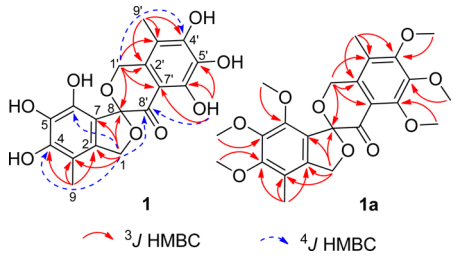
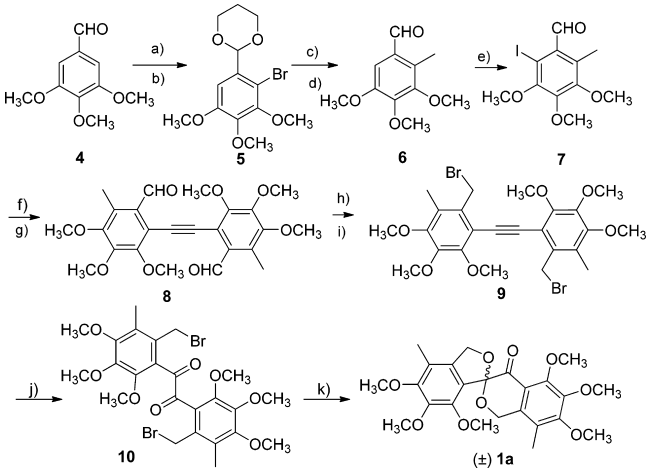


Figure 1. Key HMBC correlations of **1** and **1a**.

subunit was deduced by HMBC correlations from H-1 (δ_H 5.05, 4.93) to C-2 (δ_C 131.0), C-7 (δ_C 115.2), C-8, and C-8′, from H-1′ (δ_H 5.00, 4.81) to C-2′ (δ_C 131.4), C-7′ (δ_C 106.2), and C-8, and from OH-6′ (δ_H 11.84) to C-6′ (δ_C 150.0), C-7′, and C-8′, together with the chemical shifts of C-1 (δ_C 72.6), C-8 (δ_C 107.5), and C-1′ (δ_C 60.4). The two methyls were located at C-3 and C-3′ according to the HMBC correlations from H_3-9 (δ_H 1.98) to C-2, C-3 (δ_C 107.6), and C-4 (δ_C 146.3) and from H_3-9′ (δ_H 1.95) to C-2′, C-3′ (δ_C 111.0), and C-4′ (δ_C 152.5). According to the molecular formula, five hydroxy groups remained. Finally, the structure of **1** was established by assigning the hydroxy groups to C-4, C-5 (δ_C 132.6), C-6 (δ_C 139.7), C-4′, and C-5′ (δ_C 130.2) according to the chemical shifts[9,10] and HMBC correlations of the methoxy groups to the corresponding carbons in the permethylated product **1a** (Figure 1).

The appearance of several key ^4J HMBC correlations (Figure 1) led to the ambiguity in the structure elucidation, and compound **1** was quite unstable. In order to confirm the planar structure and determine the absolute configuration of **1** unambiguously, the total synthesis of the permethylated racemic derivate (±)-**1a** was carried out in 11 steps starting from 3,4,5-trimethoxybenzaldehyde (**4**) (Scheme 1). The synthesis started with the bromination of the 3,4,5-trimethoxybenzaldehyde in the presence of a catalytic amount of acetic acid at room temperature (rt). The resultant aldehyde was protected with 1,3-propanediol, affording the acetal **5**, which underwent methylation followed by deprotection to give **6** in 68% yield. Iodination of **6** gave the precursor **7** of the cross-coupling in 80% yield. The reaction of **7** with (trimethylsilyl)-acetylene was carried out by a Pd/Cu-catalyzed Sonogashira cross-coupling. After desilylation of the initial aryl-(trimethylsilyl)acetylene derivative, the symmetrical diarylalkyne **8** was prepared in a second Sonogashira coupling with **7**. The dibromide derivative **9** could be generated in a reduction and bromination sequence from **8**. Oxidation of **9** in the presence of $RuCl_3$ and KIO_4 furnished diketone **10** in 55% yield. With the key precursor **10** in hand, we were able to induce cyclization with KOH to get the desired ketal quaternary carbon center, and (±)-**1a** was obtained with a total yield of 7%. The identical ^1H and ^{13}C NMR data of the natural derivate **1a** and the racemic synthetic derivative (±)-**1a** further confirmed the planar structure of compound **1**.

Scheme 1. Synthesis of Compound (±)-1a[a]



[a]Reagents and conditions: (a) Br_2, AcOH, DCM (89%); (b) 1,3-propanediol, *p*-TsOH, toluene (95%); (c) *n*-BuLi, MeI, THF; (d) saturated citric acid (68% for c and d); (e) CF_3COOAg, I_2, $CHCl_3$ (80%); (f) i. TMSA, $Pd(PPh_3)_4$, CuI, TEA. ii. K_2CO_3, MeOH (57% for two steps); (g) $Pd(PPh_3)_4$, CuI, **7**, TEA (82%); (h) $NaBH_4$, MeOH, rt. (98%); (i) PBr_3, THF (99%); (j) $RuCl_3$, KIO_4, H_2O, CCl_4, MeCN (55%); (k) KOH, THF/H_2O, reflux (57%). TMSA: (Trimethylsilyl)acetylene.

The enantiomers of the synthetic racemic (±)-**1a** were separated by HPLC using a chiral stationary phase (Chiralpak ADH) (Figure 2). The enantiomer of **1a** that eluted second showed the same negative sign for the specific rotation as the natural derivative (−)-**1a**. For the determination of the absolute configuration, the solution ECD spectra of the two enantiomers were compared with the computed TDDFT-ECD spectrum of the randomly selected conformer (8*S*)-**1a** (Figure 3), which showed a similar ECD curve to the first-eluting (+)-**1a** with positive Cotton effects (CE) at 358 and 267 nm and negative ones at 312, 229, and 202 nm.[11] The MMFF conformational search of the arbitrarily selected (8*S*)-**1a** followed by DFT reoptimization at the B3LYP/6-31G(d) level afforded eight conformers above 2% population (Figure S33, Supporting Information). In all of the conformers, the isochroman ring adopted conformations with an axial C-8−O (isobenzofuran) bond and *P*-helicity [positive $\omega_{C\text{-}7',C\text{-}8',C\text{-}8,O(isochroman)}$ dihedral

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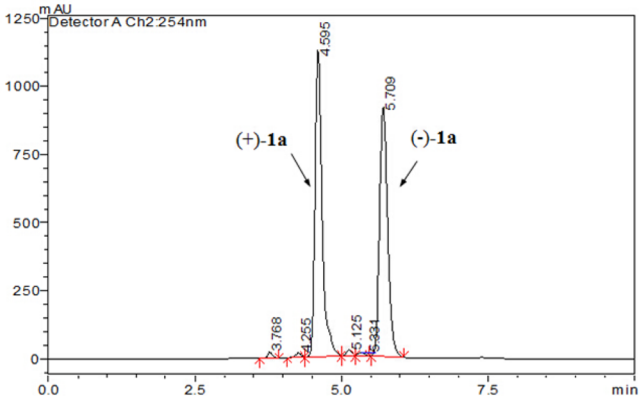

Figure 2. HPLC profile for the chiral-phase separation of (±)-**1a**.

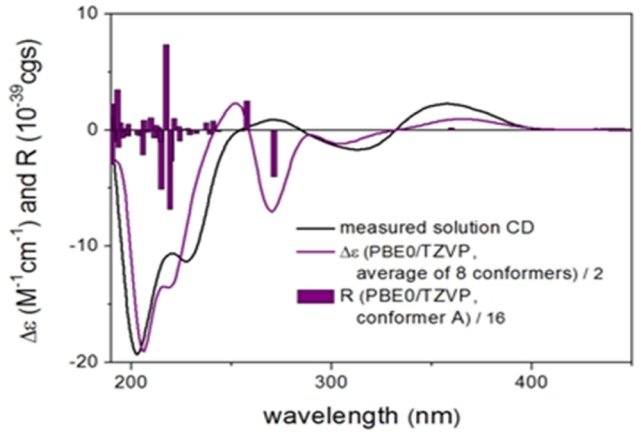

Figure 3. Experimental ECD spectrum (black) of (+)-**1a** (first-eluting enantiomer) compared with the PBE0/TZVP-calculated ECD spectrum of (8*S*)-**1a** (purple). Bars represent computed rotational strengths of the lowest energy conformer.

angle]. The conformers differed in the conformation of the fused pyran ring and orientations of the methoxy groups. The fused pyran ring of the lowest energy conformers (conformers A and B with 35.2% and 19.8% populations, Figure S33) had a half-chair conformation, which is also represented by conformers E, F, and G with a total population of 63.0% (structure I in Figure 4). Conformers C, D, and H with a total population

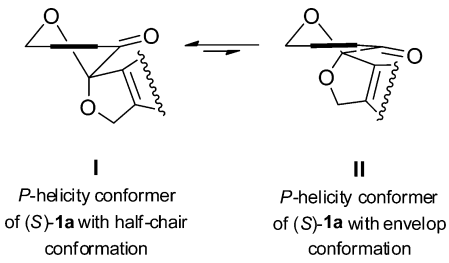

Figure 4. Equilibrating conformers of (*S*)-**1a** with half-chair and envelope conformations of the isochroman-4-one chromophore.

of 26.7% had an envelope conformation of the pyran ring (structure II in Figure 4), in which C-8 moved into the plane of the fused benzene ring. The isochroman-4-one chromophore of **1a** belongs to the group of cyclic aryl ketones such as the chroman-4-one chromophore in flavanones,[12] 2-hydroxyflavanones,[12] 2-alkylchromanones,[13] and isoflavanones[14,15] or the 2,3-dihydroquinolin-4(1*H*)-one chromophore in the recently isolated brocaeloid A.[16] The sign of the high-wavelength n−π* CE of these chromophores was correlated with the helicity of their heterocyclic ring by helicity rules, according to which *P*-helicity of the heterocyclic ring adopting an envelope or half-chair conformation is manifested in a positive n−π* CE above 300 nm.[17] Because the isochroman-4-one chromophore is an analogue of the above chromophores, a similar helicity rule is expected between the sign of the n−π* CE and the helicity of the heterocyclic ring of the isochroman-4-one moiety. Our conformational analysis revealed that the isochroman-4-one ring of (8*S*)-**1a** has *P*-helicity, which should result in a positive n−π* CE in accordance with the observed positive CE for the highest wavelength ECD band of (+)-**1a**. TDDFT-ECD spectra were calculated for low-energy conformers above 2% population with various functionals (B3LYP, BH&HLYP, PBE0) and the TZVP basis set to confirm the above configurational assignment by calculations. The computed ECD spectra of all of the conformers showed a positive CE for the lowest energy ECD transition (358 nm), which could be assigned to the n−π* transition (see Khon−Sham orbitals in Figure S34, Supporting Information). The 312 nm band was assigned to the π−π* transition of the aromatic rings. In contrast to the lowest energy ECD transition, the conformers with half-chair and envelope conformations of the isochroman-4-one ring had markedly different ECD curves in the higher energy region. Except for a negative computed CE at 270 nm, the Boltzmann-weighted ECD spectra of (8*S*)-**1a** reproduced well the experimental ECD spectrum of (+)-**1a**, confirming its absolute configuration as 8*S*. Thus, the absolute configuration of the natural **1** and **1a**, which had opposite configuration of that of (+)-**1a**, was unambiguously determined as 8*R*. The TDDFT-ECD calculations also confirmed that the same correlation exists between the helicity of the isochroman-4-one chromophore and the sign of the n−π* ECD transition as those established for the chromanone and 2,3-dihydroquinolin-4(1*H*)-one chromophores; a positive n−π* CE derives from *P*-helicity.

The synthesized (±)-**1a** and its separated enantiomers showed no cytotoxicity against K562 and HL-60 cells (IC_{50} > 50 μM). The antiviral activities of synthesized (+)-**1a** and (−)-**1a** were also evaluated against influenza A virus (H_1N_1) in the cytopathic effect (CPE) inhibition assay.[18] Only compound (−)-**1a** exhibited activity with an IC_{50} = 149 μM against the influenza A H1N1 virus (ribavirin was used as positive control, IC_{50} 101 μM).

Eleganketal A (**1**) is the first naturally occurring polyketide having a spiro[isobenzofuran-1,3′-isochroman] ring system, which has been reported in only a few synthetic molecules.[19] Structurally, crombenine, which was isolated from the plant *Acacia crombei* C. T. White, is the most similar natural product to eleganketal A.[20] Crombenine differs from compound **1** by possessing a spiro[benzofuran-2,3′-isochroman] ring system, and the absolute configuration was undetermined. The total synthesis of crombenine has been attempted, but only a bicyclic [4.3.1] derivative was formed.[21] The highly substituted aromatic rings, lack of proton signals, and low stability of **1** exerted difficulties in determining the structure by spectroscopic methods, especially the absolute configuration. In this work, we determined the structure of **1** by a combination of spectroscopic analysis, chemical synthesis of the permethylated analogue, chiral-phase HPLC separation of a synthetic analogue, and TDDFT ECD calculations.

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EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. CD spectra were measured on a JASCO J-715 spectropolarimeter. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer in KBr discs. ^{1}H NMR, ^{13}C NMR, and DEPT spectra and 2D NMR were recorded on JEOL JNM-ECP 600, Agilent 500 MHz DD2, and Bruker AM-400 spectrometers using TMS as internal standard. ESIMS utilized a Waters Q-TOF Ultima Global mass spectrometer and a Thermo Scientific LTQ Orbitrap XL mass spectrometer. Semipreparative HPLC was performed using an ODS column [HPLC (YMC-Pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min)]. Racemic mixtures were resolved on a Chiralpak ADH column (5 μm, 4.6 × 250 mm, hexane/2-propanol eluent, 1 mL/min). TLC and column chromatography (CC) were performed on plates precoated with silica gel GF254 (10−40 μm) and over silica gel (200−300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory). All starting materials, reagents, and solvents were purchased from commercial sources and used as such without further purification: AcOH, MeCN, DCM, MeOH, sodium borohydride ($NaBH_4$), sodium bicarbonate (Na_2CO_3), sodium chloride (NaCl), sodium sulfate (Na_2SO_4), sodium thiosulfate ($Na_2S_2O_3$), tetrachloromethane (CCl_4), tetrahydrofuran (THF), toluene ($PhCH_3$), triethylamine (Et_3N), petroleum ether (PE). Unless otherwise stated, all reactions were carried out using anhydrous solvents. The seawater was collected from Jiaozhou Bay, Qingdao, China.

Fermentation, Extraction, and Isolation of Compound 1. The fungal strain[4] *S. elegans* KLA03 was cultured with shaking in 500 mL Erlenmeyer flasks each containing 150 mL of liquid medium (mannitol 2.0%, maltose 2.0%, glucose 1.0%, NH_4Cl 1.0%, KH_2PO_4 0.05%, $MgSO_4{\cdot}7H_2O$ 0.03%, yeast extract 0.3%, corn steep liquor 0.1%, and naturally collected seawater after adjusting pH to 6.5) at 28 °C. After 8 days of cultivation, 20 L of whole broth was extracted with EtOAc (10 L × 3) and concentrated under reduced pressure to give a dark brown gum. The EtOAC extract (20 g) was subjected to vacuum liquid chromatography over a silica gel (200−300 mesh) column using stepwise gradient elution with mixtures of petroleum ether/$CHCl_3$/MeOH to give five fractions. Fraction 3 was rechromatographed on a silica gel column, eluting with a petroleum ether/acetone gradient, to provide 10 subfractions (fractions 3.1−3.10). Fraction 3.10 was further purified on Sephadex LH-20 (MeOH) to give compound **1** (8.0 mg).

Eleganketal A (1): yellow, amorphous powder; $[\alpha]^{20}_D$ −90 (*c* 0.10, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 375.0710 $[M - H]^-$ (calcd for $C_{18}H_{15}O_9$, 375.0716).

Preparation of the Methylated Derivative 1a from 1. Eleganketal A (**1**, 4.0 mg) was stirred with iodomethane (15 μL) and potassium carbonate (10 mg) in refluxing anhydrous acetone overnight. The reaction mixture was filtered and washed with acetone. The acetone solution was evaporated under vacuum and further purified on HPLC (t_R = 9.8 min, 75% MeOH/H_2O) to afford **1a** (1 mg): pale yellow oil; $[\alpha]^{20}_D$ −50 (*c* 0.10, MeOH); ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 5.21 (1H, d, *J* = 15.8 Hz; H-1), 5.20 (1H, d, *J* = 12.4 Hz; H-1′), 5.09 (1H, d, *J* = 12.4 Hz; H-1′), 4.89 (1H, d, *J* = 15.8 Hz; H-1), 3.95 (3H, s; 4′-$OCHH_3$), 3.91 (6H, s; 4-$OCHH_3$ and 6′-$OCHH_3$), 3.88 (3H, s; 5-$OCHH_3$), 3.87 (3H, s; 6-$OCHH_3$), 3.85 (3H, s; 5′-$OCHH_3$), 2.09 (3H, s; H-9′); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 186.0 (C-8′), 156.8 (C-4′), 154.4 (C-4′), 154.3 (C-4), 146.8 (C-6), 146.1 (C-5), 145.9 (C-6′), 136.7 (C-3′), 135.2 (C-3), 125.1 (C-7), 122.0 (C-2′), 119.7 (C-2), 118.8 (C-7′), 108.9 (C-8), 73.4 (C-1), 61.9 (6′-$OCHH_3$), 61.7 (OCH_3-4), 61.4 (6-$OCHH_3$), 61.0 (5-$OCHH_3$ and 4′-$OCHH_3$), 60.9 (C-1′ and 5′-$OCHH_3$), 12.4 (C-9′), 10.7 (C-9); LRESIMS $[M + H]^+$ *m/z* 461.

Total Synthesis of (±)-1a. Preparation of 2-Bromo-3,4,5-trimethoxybenzaldehyde. 3,4,5-Trimethoxybenzaldehyde (19.60 g, 0.10 mol, 1.0 equiv) was dissolved in 200 mL of CH_2Cl_2 followed by adding 500 μL of AcOH. After the addition of bromide (5.64 mL, 0.11 mol, 1.1 equiv) dissolved in 50 mL of CH_2Cl_2, the mixture was stirred at rt for 30 min. The reaction solution was washed with a saturated aqueous $Na_2S_2O_3$ solution. The organic layer was dried over anhydrous Na_2SO_4 and purified by CC (EtOAc/PE = 1:20) to afford 2-bromo-3,4,5-trimethoxybenzaldehyde as white crystals with a yield of 89%: mp 65−66 °C; ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 10.28 (1H, s), 7.29 (1H, s), 3.97 (3H, s), 3.90 (3H, s), 3.89 (3H, s); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 191.2, 153.1, 150.9, 148.8, 128.9, 115.7, 107.5, 61.4, 61.3, 56.4; ESIMS (*m/z*) 275/277 $[M + H]^+$.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for 1 in DMSO-d_6

position	δ_C (type)	δ_H mult (*J* in Hz)
1	72.6, CH_2	5.05, d (12.0); 4.93, d (12.0)
2	131.0, C	
3	107.6, C	
4	146.3, C	
5	132.6, C	
6	139.7, C	
7	115.2, C	
8	107.5, C	
9	11.9, CH_3	1.98, s
1′	60.4, CH_2	5.00, d (15.6); 4.81, d (15.6)
2′	131.4, C	
3′	111.0, C	
4′	152.5, C	
5′	130.2, C	
6′	150.0, C	
7′	106.2, C	
8′	192.2, C	
9′	9.7, CH_3	1.95, s
6′-OH		11.84, s

Preparation of 2-(2-Bromo-3,4,5-trimethoxyphenyl)-1,3-dioxane (5). The 2-bromo-3,4,5-trimethoxylbenzaldehyde (5.48 g, 20.00 mmol, 1.0 equiv), 1,3-propanediol (4.57 g, 60.00 mmol, 3.0 equiv), and a catalytic amount of *p*-toluene sulfonic acid were dissolved in 200 mL of toluene in a 250 mL round-bottom flask equipped with a Dean−Stark apparatus. The solution was heating to reflux for 24 h. After cooling to rt, the mixture was washed with saturated Na_2CO_3 solution three times, and the organic layer was dried over anhydrous Na_2SO_4. Toluene was removed to give compound **5** as a colorless oil (6.35 g; 95% yield): ^{1}H NMR NMR δ_H (600 MHz, $CDCl_3$) 7.08 (1H, s), 5.75 (1H, s), 4.24−4.27 (2H, dd), 4.00−4.04 (2H, td), 3.89 (3H, s), 3.87 (3H, s), 3.85 (3H, s), 2.23 (1H, m), 1.50 (1H, m); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 153.1, 150.7, 143.7, 133.1, 109.1, 106.6, 100.9, 67.7, 61.2, 56.2, 25.7; MSESI (*m/z*) 333/335 $[M + H]^+$.

Preparation of 3,4,5-Trimethoxy-2-methylbenzaldehyde (6). Compound **5** (3.33 g, 10.00 mmol, 1.0 equiv) was dissolved with 100 mL of freshly redistilled anhydrous THF in a two-necked round-bottom flask under an atmosphere of argon. After cooling to −78 °C, 1.6 N *n*-butyllithium (9.38 mL, 15.00 mmol, 1.5 equiv) was added through a syringe. After stirring for 1 h, methyl iodide (935.0 μL, 15.00 mmol, 1.5 equiv) was added dropwise. The reaction mixture was slowly warmed to rt and stirred overnight. The reaction was quenched with saturated aqueous citric acid solution, washed with EtOAc three times, and dried over anhydrous Na_2SO_4. Compound **6** was purified by CC (EtOAc/PE = 1:8) and was obtained as a yellow oil (1.42 g; 68% yield): ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 10.24 (1H, s), 7.20 (1H, s), 3.95 (3H, s), 3.89 (3H, s), 3.83 (3H, s), 2.51 (3H, s); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 190.9, 152.2, 151.7, 147.9, 129.5, 128.8, 108.3, 61.0, 60.9, 56.1, 10.2; MSESI (*m/z*) 211 $[M + H]^+$.

Preparation of 2-Iodo-3,4,5-trimethoxy-6-methylbenzaldehyde (7). Compound **6** (3.00 g, 14.28 mmol, 1.0 equiv) was dissolved in 100 mL of $CHCl_3$ followed by the addition of 3.63 g of CF_3COOAg (16.42 mmol, 1.15 equiv). To this suspension was added dropwise over 30 min 3.63 g of iodine (14.28 mmol, 1.0 equiv)

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dissolved in 150 mL of $CHCl_3$. The silver iodide formed was filtered away, and the organic layer was washed by a saturated solution of $Na_2S_2O_3$ and brine. After drying over anhydrous Na_2SO_4, the crude product was purified by CC (EtOAc/PE = 1:10), and **7** was obtained as yellow crystals with a yield of 80% (3.84 g): ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 10.20 (1H, s), 3.98 (3H, s), 3.87 (3H, s), 3.81 (3H, s), 2.42 (3H, s); MSESI (*m*/*z*) 337 $[M + H]^+$.

Preparation of 2-Ethynyl-6-methyl-3,4,5-trimethoxybenzaldehyde. To an oven-dried 50 mL round-bottomed flask were added compound **7** (0.60 g, 1.80 mmol) and anhydrous TEA (18.00 mL). After 20 min of purging with nitrogen, CuI (34.9 mg, 0.18 mmol) and $Pd(PPh_3)_4$ (72.5 mg, 0.063 mmol) were added. Trimethylsilylacetylene (0.50 mL, 3.60 mmol) was added very slowly, and the mixture was stirred at 60 °C for 20 h. After cooling and filtration, a saturated NaCl solution was added, and the mixture was extracted with EtOAc three times. The organic layer was dried by anhydrous Na_2SO_4, and EtOAc was removed under vacuum. The 3,4,5-trimethoxy-2-methyl-6-((trimethylsilyl)ethynyl)benzaldehyde was dissolved in MeOH (20 mL), and oven-dried K_2CO_3 (0.26 g, 1.90 mmol) was added in one portion. The flask was placed under a nitrogen atmosphere, and the mixture was stirred for 15 min. Then H_2O was added to the solution, which was extracted with EtOAc three times. After concentration in vacuo, 2-ethynyl-6-methyl-3,4,5-trimethoxybenzaldehyde was purified by CC (EtOAc/PE = 1:10) and obtained as a white snowflake solid (0.24 g) with a yield of 57%: mp 64−65 °C; ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 10.61 (1H, s), 3.98 (3H, s), 3.94 (3H, s), 3.82 (3H, s), 3.63 (1H, s), 2.46 (3H, s); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 193.1, 154.0, 153.1, 150.7, 131.2, 131.1, 118.0, 88.3, 75.6, 61.6, 61.1, 61.0, 12.4; MSESI (*m*/*z*) 235 $[M + H]^+$.

Preparation of 6,6′-(Ethyne-1,2-diyl)bis(3,4,5-trimethoxy-2-methyl benzaldehyde) (8). Compound **7** (148.0 mg, 0.44 mmol) was added to the TEA solution of $Pd(PPh_3)_4$ (25.0 mg) and CuI (5.0 mg) at rt, and the mixture was stirred for 15 min under the protection of nitrogen. The TEA (5.0 mL) solution of 2-ethynyl-6-methyl-3,4,5-trimethoxybenzaldehyde (93.6 mg, 0.4 mmol) was added by syringe over 2 min. The mixture was heated to 60 °C and stirred for 10 h. During the reaction process, a solid precipitated. After cooling to rt, the solid was filtered and washed with PE and H_2O three times, respectively, which led to recovery of compound **7** as a white solid (158.9 mg; yield 82%): mp 194−195 °C; ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 10.79 (2H, s), 4.01 (6H, s), 3.99 (6H, s), 3.85 (6H, s), 2.51 (6H, s); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 193.3, 153.4, 153.2, 150.8, 131.2, 130.5, 119.0, 92.3, 61.6, 61.2, 61.1, 12.4; MSESI (*m*/*z*) 443 $[M + H]^+$.

Preparation of 6,6′-(Ethyne-1,2-diyl)bis(3,4,5-trimethoxy-2-methylbenzyl alcohol). $NaBH_4$ (68.8 mg, 1.81 mmol, 4 equiv) was added to the solution (5.0 mL MeOH) of compound **8** (0.2 g, 0.45 mmol), and the mixture was stirred for 8 h. After removal of MeOH, 10.0 mL of H_2O was added and filtration provided a white solid of 6,6′-(ethyne-1,2-diyl)bis(3,4,5-trimethoxy-2-methylbenzyl alcohol) (792.0 mg; yield 98%): mp 138−139 °C; ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 4.88 (4H, s), 4.01 (6H, s), 3.91 (6H, s), 3.85 (6H, s), 2.31 (6H, s); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 153.0, 152.9, 145.8, 136.80, 127.1, 113.8, 91.4, 61.5, 61.1, 60.9, 60.7, 11.7; MSESI (*m*/*z*) 447 $[M + H]^+$.

Preparation of 6,6′-(Ethyne-1,2-diyl)bis(3,4,5-trimethoxy-2-methylbenzyl bromide) (9). The 6,6′-(ethyne-1,2-diyl)bis(3,4,5-trimethoxy-2-methylbenzyl alcohol) (0.98 g, 0.22 mmol) was dissolved in 10 mL of anhydrous THF under a nitrogen atmosphere and was cooled in an ice bath. After the addition of phosphorus tribromide (0.90 mmol, 4.0 equiv), the solution was stirred at rt for 1 h. Removing THF under vacuum gave the compound **9** as a white solid (124.5 mg) with a yield of 99%: mp 167−168 °C; ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 4.91 (4H, s), 4.05 (6H, s), 3.93 (6H, s), 3.87 (6H, s), 2.31 (6H, s); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 153.2, 153.0, 146.8, 133.2, 127.8, 114.6, 91.4, 61.9, 61.0, 60.9, 31.0, 11.7; MSESI (*m*/*z*) 570/572/574 $[M + H]^+$.

Preparation of 1,2-Bis(2-(bromomethyl)-4,5,6-trimethoxy-3-methylphenyl)ethan-1,2-dione (10). To a solution of compound **9** (0.19 g, 0.33 mmol, 1.0 equiv) in a mixture of CCl_4, MeCN, and H_2O (8:8:1, v/v, 20 mL) was added KIO_4 (0.31 g, 1.35 mmol, 4.1 equiv) followed by $RuCl_3$ (1.5 mg, 7.25 μmol, 0.022 equiv). After vigorously stirring for 1 h at rt, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2 three times. The organic layer was collected and dried by anhydrous Na_2SO_4. The crude compound **10** was purified by CC (EtOAc/PE = 1:10) and obtained as a white snowflake solid with a yield of 55%: mp 156−157 °C; ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 4.94 (4H, s), 3.91 (6H, s), 3.83 (6H, s), 3.69 (6H, s), 2.32 (6H, s); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 190.9, 156.8, 154.4, 145.7, 134.6, 128.7, 121.6, 60.9, 60.6, 58.5, 28.4, 11.0; MSESI (*m*/*z*) 602/604/606 $[M + H]^+$.

Preparation of the Racemic Methylated Eleganketal (±)-1a. Compound **10** (0.10 g, 0.17 mmol) was dissolved in a mixture of 4 mL of THF and 1 mL of H_2O. The mixture was stirred under reflux for 24 h after addition of KOH (93.0 mg, 1.66 mmol, 10 equiv). The solution was diluted with 20 mL of H_2O and extracted with EtOAc three times. The organic layer was collected, washed with brine, and dried over anhydrous Na_2SO_4. The crude product was purified by CC (EtOAc/PE = 1:8) to give the pure target compound (44.5 mg) as a colorless oil with a yield of 57%: ^{1}H NMR δ_H (600 MHz, $CDCl_3$) 5.193 (1H, d, *J* = 16.0 Hz), 5.189 (1H, d, *J* = 12.1 Hz), 5.07 (1H, d, *J* = 12.1 Hz), 4.88 (1H, d, *J* = 15.4 Hz), 3.93 (3H, s), 3.90 (6H, s), 3.87 (3H, s), 3.86 (3H, s), 3.84 (3H, s), 2.08 (3H, s); ^{13}C NMR δ_C (150 MHz, $CDCl_3$) 186.0, 156.8, 154.4, 154.2, 146.8, 146.1, 145.9, 136.7, 135.2, 125.1, 122.0, 119.7, 118.8, 108.9, 73.4, 61.9, 61.7, 61.3, 61.0, 60.9, 12.4, 10.7; HRESIMS (*m*/*z*) 461.1808 $[M + H]^+$ (calcd for $C_{24}H_{29}O_9$, 461.1806).

Chiral-Phase Separation of the (±)-1a. The racemic (±)-**1a** (27.0 mg) was injected onto a chiral-phase (CHIRALPAK ADH) HPLC column. Eluting with hexane/2-propanol (70/30), the two enantiomers (+)-**1a** (9.1 mg) and (−)-**1a** (14.4 mg) were obtained, with rotation values (*c* 0.10, MeOH) of +40 and −40, respectively. ECD (2.23×10^{-4} M, MeCN) of (−)-**1a**: λ_{max} ($\Delta\varepsilon$) 358 (2.25), 312 (−1.75), 267 (0.88), 229 sh (−12.29), 202 (−21.62).

Computational Section. Mixed torsional/low mode conformational searches were carried out by means of the Macromodel 9.9.223[22] software using the Merck molecular force field (MMFF) with an implicit solvent model for chloroform applying a 21 kJ/mol energy window. The resultant 56 conformers were reoptimized at the B3LYP/6-31G(d) level in vacuo. DFT optimizations and TDDFT calculations were performed with Gaussian 09[23] using various functionals (B3LYP, BH&HLYP, PBE0) and the TZVP basis set. ECD spectra were generated as the sum of Gaussians with 1800 cm^{-1} half-height width (corresponding to ∼7 at 200 nm), using dipole-velocity-computed rotational strengths.[24] Boltzmann distributions were estimated from the ZPVE-corrected B3LYP/6-31G(d) energies. The MOLEKEL[25] software package was used for visualization of the results.

Anti-influenza A (H1N1) Assay. The antiviral activity against H1N1 was evaluated by the CPE inhibition assay. Confluent MDCK cell monolayers were incubated with influenza virus (A/Puerto Rico/8/34 (H1N1), PR/8) at 37 °C for 1 h. After removing the virus dilution, cells were maintained in infecting media (RPMI 1640, 4 μg/mL of trypsin) containing different test compounds at 37 °C. After 48 h incubation at 37 °C, the cells were fixed with 100 μL of 4% formaldehyde for 20 min at rt. After removal of the formaldehyde, the cells were stained with 0.1% crystal violet for 30 min. The plates were washed and dried, and the intensity of crystal violet staining for each well was measured in a microplate reader (Bio-Rad) at 570 nm. The IC_{50} was calculated as the compound concentration required to inhibit influenza virus yield at 48 h postinfection by 50%. Ribavirin was used as the positive control in the CPE inhibition assay.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR and MS spectra of **1** and **1a**; NMR spectra of the key intermediates for the total synthesis of (±)-**1a**. These materials are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: 0086-532-82031619. Fax: 0086-532-82033054. E-mail: dehaili@ouc.edu.cn.

Notes

The authors declare no competing financial interest.

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