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Total synthesis of dehydroaltenusin

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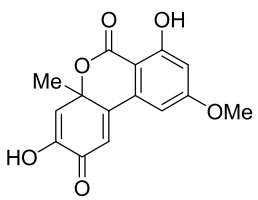
Abstract—The first total synthesis of dehydroaltenusin, a natural enzyme inhibitor, is described. The key step involves Suzuki-coupling reaction of an aryl triflate prepared from 2,4,6-trihydroxybenzoic acid with a catechol-derived boronic acid or boronic ester. The synthetic product was evaluated as a potent inhibitor against eukaryotic DNA polymerase α and other DNA polymerases.

1. Introduction

Eukaryotic multicellular organisms are known to contain at least 14 types of DNA polymerases.[1,2] DNA polymerase α is an essential enzyme for DNA replication and subsequently for cell division.[1] Aphidicolin, a well-known DNA polymerase α inhibitor, has been very useful for studying the DNA replication system,[3] however, there have been no previous reports of inhibitors capable of distinguishing among DNA polymerases α, δ and ϵ. Recently, we have isolated a powerful mammalian DNA polymerase α inhibitor (IC_{50}=0.68 μM) from *Acremonium* sp. 98H02B04-1 (2) and revealed it to be dehydroaltenusin (**1**),[4] which was discovered from mycelium extracts of *Alternaria tennuis* and *A. kikuchiana* by Rosett et al. in 1957[5] and then from a variety of fungi.[6,7] The structure was initially suggested to be a γ-lactone derivative of β-resorcylic acid monomethylether based on the chemical and spectroscopic data[6] and later revised to **1** possessing a δ-lactone ring by the X-ray crystallographic analyses (Fig. 1).[8] In 1995, **1** was also reported to inhibit the calmodulin-dependent activity of myosin light chain kinase (MLCK).[9]



dehydroaltenusin (**1**)

Figure 1.

Compound **1** inhibits only mammalian DNA polymerase α activity, but does not influence the activities of mammalian DNA polymerase δ and ϵ, nor DNA polymerase α from other vertebrates in vitro, and found to be more potent inhibitor of DNA polymerase α than aphidicolin.[4] We have also reported **1** suppressed the cell proliferation of the human gastric cell line NUGC-3 by inhibiting DNA polymerase α activity.[10] The specific inhibitors of mammalian DNA polymerase α are not only molecular tools and molecular probes to distinguish DNA polymerases and clarify their biological and in vivo functions, but should also be considered as a group of potentially useful cancer chemotherapy agents. However, its low producibility has prevented such utilization. Furthermore, no total synthesis of **1** has been reported so far. We report herein the first synthesis of racemic **1** and its inhibitory activity against a series of DNA polymerases.[11]

2. Results and discussion

2.1. Total synthesis of dehydroaltenusin

Rosett et al. has reported that $FeCl_3$-promoted oxidation of altenusin **2** afforded **1**.[5] Therefore, **2** was regarded as our

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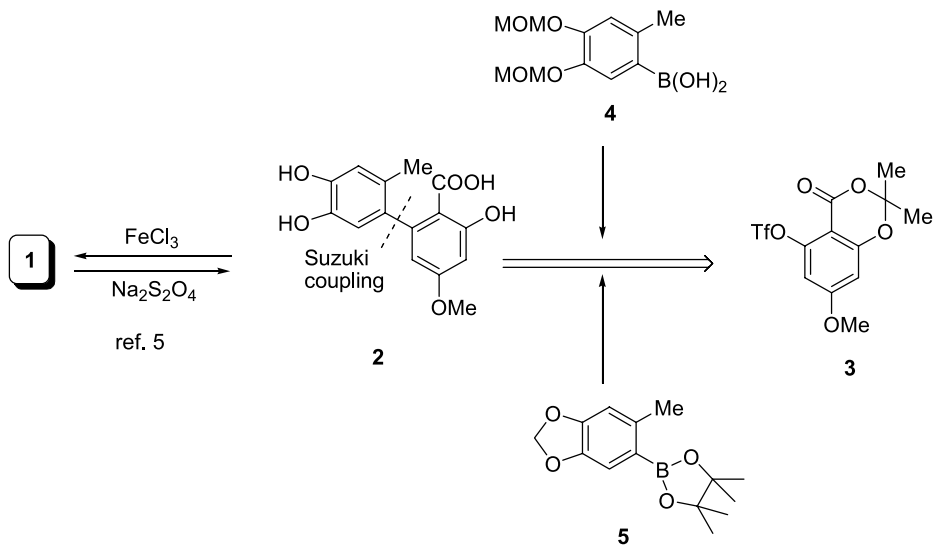
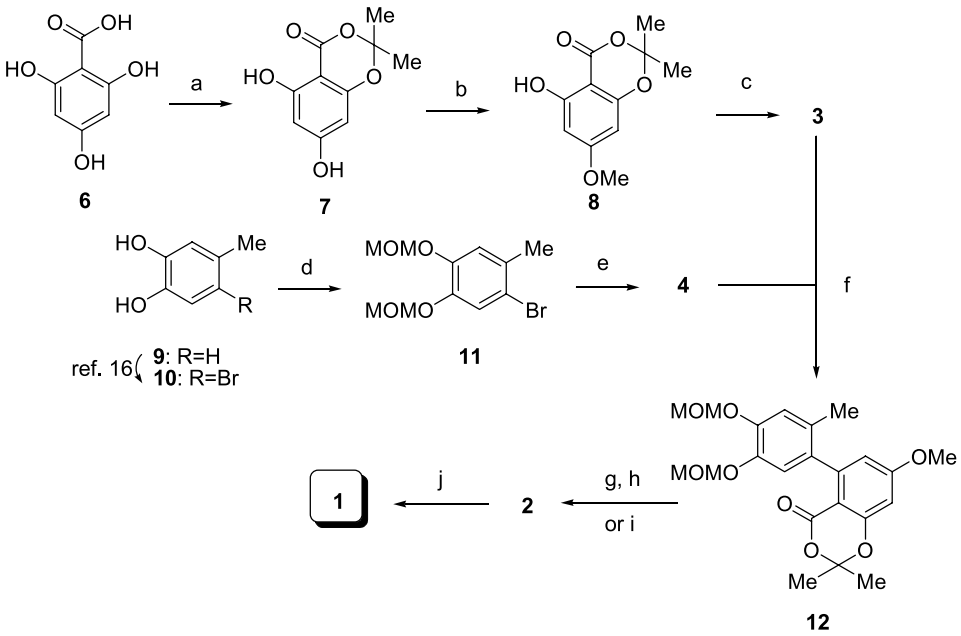


Scheme 1. Retrosynthetic scheme of dehydroaltenusin (**1**).

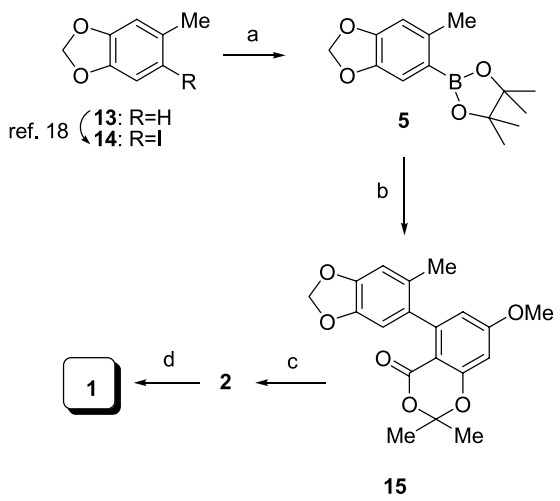


Scheme 2. Reagents and conditions: (a) acetone, $SOCl_2$, DMAP, DME, rt, 56%; (b) DIAD, Ph_3P, MeOH, THF, rt, 89%; (c) Tf_2O, pyridine, 0 °C, 94%; (d) MOMCl, NaH, DMF, 0 °C, 90%; (e) *n*-BuLi, THF, −78∼−40 °C, then $(i\text{-PrO})_3B$, Et_2O, −78 °C∼rt, 95%; (f) $(Ph_3P)_4Pd$, K_3PO_4, KBr, dioxane, 100 °C, 93%; (g) 2N KOH, EtOH, 60 °C; (h) 10% HCl–MeOH, CH_2Cl_2, rt, 64% (two steps); (i) BCl_3 (10 equiv.), CH_2Cl_2, 0 °C∼rt, 63%; (j) $FeCl_3$, aq. EtOH, rt, 82%.

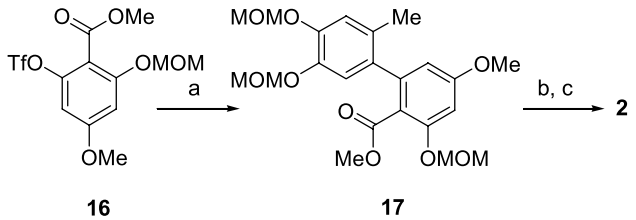
actual synthetic goal. Our synthetic efforts toward **2** involved Suzuki-coupling[12] reaction of an aryltriflate **3** with an aryl boronic acid **4** or boronic ester **5** as a key step (Scheme 1). Synthesis of the aryltriflate **3** started from commercially available 2,4,6-trihydroxybenzoic acid **6**. The benzoic acid **6** was reacted with thionyl chloride ($SOCl_2$) in the presence of *N*,*N*-dimethylaminopyridine (DMAP) in acetone[13] to give acetonide **7** in 56% yield, whereas in 43% yield by Danishefsky's method (trifluoroacetic acid–trifluoroacetic anhydride in acetone)[14] (Scheme 2). Danishefsky et al. has accomplished regioselective protection of 4-hydroxy group of **7** by the Mitsunobu conditions[15] with diisopropyl azodicarboxylate–triphenylphosphine in the presence of benzyl alcohol.[14] Regioselective methylation of **7** was performed according to this method, affording monomethyl ether **8** in 89% yield. Treatment of **8** with triflic anhydride-pyridine gave the corresponding triflate **3** in 94% yield. On the other hand, a 4-bromocatechol **10**[16] prepared from 4-methylcatechol (**9**) was subjected to methoxymethylation with sodium hydride (NaH) and methoxymethyl chloride (MOMCl), giving bis-MOM ether **11** in 90% yield. Halogen–lithium exchange of **11** with *n*-butyllithium in tetrahydrofuran (THF) at −78→−40 °C followed by trapping with triisopropyl borate (Et_2O, −78 °C→rt) afforded an aryl boronic acid **4** in 95% yield. This compound was, without purification, employed to the next coupling reaction, because of its instability. Introduction of a catechol moiety into **3** was best realized by using 1.5 mol equiv. of **4** in the presence of tetrakis(triphenylphosphine)palladium (0.05 mol equiv.), potassium

phosphate and potassium bromide in dioxane[17] at 100 °C to produce a coupled product **12** in 93% yield. Alkaline hydrolysis of **12** and subsequent acid treatment provided altenusin **2** in 64% yield. This compound was also obtained by the action of boron trichloride (BCl_3) in dichloromethane (CH_2Cl_2) from **12** in a single step (63%). Finally, $FeCl_3$-promoted oxidation[5] of **2** afforded dehydroaltenusin (**1**) in 82% yield.† The spectroscopic and physical properties of **1** were identical with those of natural **1** (vide infra).

Furthermore, we have also developed an alternative shorter route through boronic ester **5** (Scheme 3). 2-Iode-4,5-methylenedioxytoluene (**14**)[18] obtained from commercially available 3,4-methylenedioxytoluene (**13**) was treated with bis(pinacolato)diboron[19] in the presence of dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium {$PdCl_2$(dppf), 0.1 mol equiv.} and potassium acetate in *N*,*N*-dimethylformamide, giving boronic ester **5** in 81% yield. Triflate **3** was coupled with the boronic ester **5** (1.3 mol equiv.) using $PdCl_2$(dppf) (0.05 mol equiv.) and potassium carbonate (3.0 mol equiv.) in 1,2-dimethoxyethane (DME)[20] at 85 °C to provide **15** in 67% yield. Deprotection of **15** by BCl_3 yielded **2**, which was transformed into dehydroaltenusin (**1**).



Scheme 3. Reagents and conditions: (a) bis(pinacolato)diboron, $PdCl_2$-(dppf), KOAc, DMF, 80 °C, 81%; (b) **3**, $PdCl_2$(dppf), K_2CO_3, DME, 85 °C, 67%; (c) BCl_3, CH_2Cl_2, rt, 80%; (d) $FeCl_3$, aq. EtOH, rt, 82%.



Scheme 4. Reagents and conditions: (a) **4**, $(Ph_3P)_4Pd$, K_3PO_4, KBr, dioxane, 100 °C, 70%; (b) 2 N KOH, EtOH, 60 °C; (c) 10% HCl–MeOH, CH_2Cl_2, rt, 86% (two steps).

† Our initial approach to **1** was based on the coupling reaction of **4** and triflate **16** ((Scheme 4)). However, we did not adopt this route for large-scale synthesis of **1** because preparation of **16** from methyl 2,6-dihydroxy-4-methoxybenzoate[23] was found to be not practical.

2.2. Inhibition studies and discussion

DNA polymerase inhibition assay was performed as described previously.[21,22] Inhibitory activity of the synthetic compound **1** against the calf DNA polymerase α, rat DNA polymerase β, calf DNA polymerase δ and human DNA polymerase ϵ was examined and compared with that of natural **1**. As illustrated in Figure 2, the IC_{50} values of synthetic and natural **1** for DNA polymerase α were 0.8 and 0.7 μM, respectively, and both compounds had no inhibitory effect on DNA polymerase β, δ and ϵ. Therefore, the inhibitory activity of the two compounds was not distinguishable.

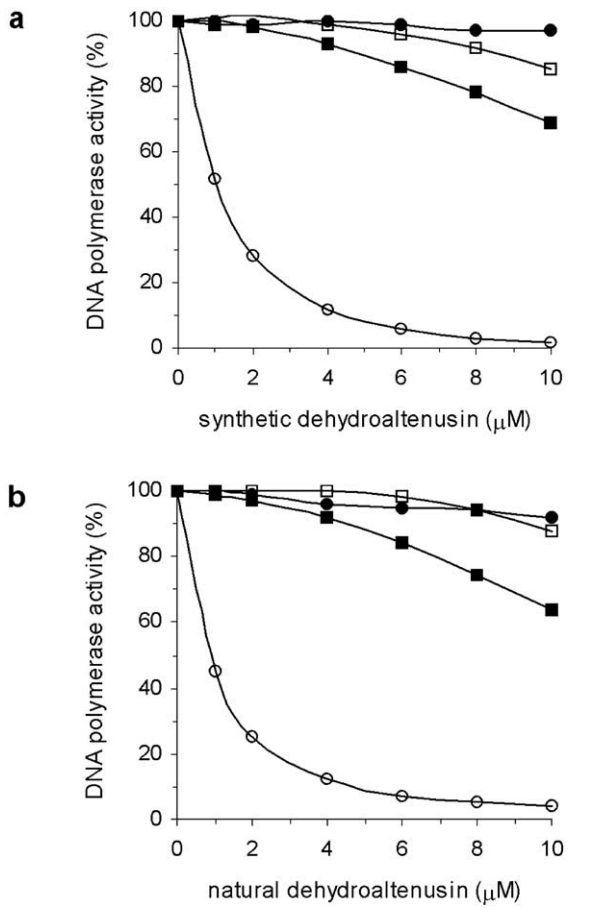


Figure 2. Dose–response curve of synthetic (a) and natural (b) dehydroaltenusin. Inhibition activity against eukaryotic DNA polymerase α, β, δ and ϵ are shown as open circle, closed circle, open square, and closed square symbols, respectively. DNA polymerase activity in the absence of compound was taken as 100%.

2.3. Conclusion

In summary, the first synthesis of dehydroaltenusin (**1**) has been accomplished in 7 steps with 23% yield or in 6 steps with 21% overall yield from a commercially available carboxylic acid **6**. These synthetic processes would be quite useful for preparation of large amounts of **1** suitable for in vitro and in vivo experiments on DNA polymerase α and future clinical usage.

3. Experimental

3.1. General procedures

^{1}H and ^{13}C NMR spectra were recorded at 400 MHz with Bruker DRX-400 spectrometer, using tetramethylsilan as the internal standard. IR spectra were recorded with a HORIBA FREEXACT-II FT-720 spectrophotometer. High-resolution mass spectra were obtained on Applied Biosystems QSTAR Mass Spectrometer using electron spray ionization (ESI) method. Column chromatography was performed on Kanto silica gel (spherical, neutral; 40–50 μm). Merck precoated silica gel 60 F_{254} 0.25 mm thickness, was used for analytical thin-layer chromatography. All reactions were performed under argon atmosphere. The solvent extracts were dried with sodium sulfate, and the solutions were evaporated under diminished pressure at 30–50 °C.

3.1.1. 5,7-Dihydroxy-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (7). To a solution of 2,4,6-trihydroxy-benzoic acid (**6**) (23.0 g, 135 mmol), *N*,*N*-dimethylaminopyridine (1.65 g, 13.4 mmol), and acetone (25.7 mL, 350 mmol) in 1,2-dimethoxyethane (100 mL) was added dropwise thionyl chloride (29 mL, 398 mmol) at 0 °C. The mixture was warmed slowly to rt and then stirred for 2 h. The mixture was poured into sat. $NaHCO_3$ solution, and extracted with EtOAc. The extracts were washed with water, dried, and concentrated. Chromatography on silica gel with hexane–EtOAc (13:7) as the eluent yielded **7** (16.0 g, 56%) as white solids: mp 200–201 °C (lit.[14] 203–204 °C); ^{1}H NMR (400 MHz, acetone-d_6): δ 1.72 (6H, s), 3.02 (1H, brs), 6.01 (1H, d, *J*=2.2 Hz), 6.08 (1H, d, *J*=2.2 Hz,), 10.46 (1H, s); ^{13}C NMR (100 MHz, acetone-d_6): δ 25.6, 93.0, 96.2, 98.0, 107.6, 158.1, 164.0, 165.8, 167.2; HRMS calcd for $C_{10}H_{10}O_5Na$ $[M+Na]^+$ 233.0420, found 233.0428.

3.1.2. 5-Hydroxy-7-methoxy-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (8). To a stirred solution of **7** (2.54 g, 12.1 mmol), methanol (0.53 mL, 13.0 mmol) and triphenylphosphine (3.40 g, 13.0 mmol) in tetrahydrofuran (40 mL) was added dropwise diisopropyl azodicarboxylate (2.6 mL, 13.0 mmol) at 0 °C, and then the mixture was stirred at 0 °C→rt for 4.5 h. The mixture was diluted with EtOAc, washed with water, brine, dried, and concentrated. Chromatography on silica gel with hexane→hexane–EtOAc (10:1) as the eluent yielded **8** (2.41 g, 89%) as white solids: mp 108–109 °C {hexane–EtOAc (20:1)}; IR (KBr) 3194, 2985, 2947, 2854, 1697, 1635, 1581, 1192, 1157 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (6H, s), 3.82 (3H, s), 6.00 (1H, d, *J*=2.3 Hz), 6.15 (1H, d, *J*=2.3 Hz), 10.45 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.6, 55.7, 93.1, 94.6, 95.7, 106.9, 156.8, 163.1, 165.2, 167.7; HRMS calcd for $C_{11}H_{12}O_5Na$ $[M+Na]^+$ 247.0576, found 247.0587. Anal. Found: C, 58.91; H, 5.51. Calcd for $C_{11}H_{12}O_5$: C, 58.93; H, 5.39.

3.1.3. 7-Methoxy-2,2-dimethyl-5-[(trifluoromethyl)-sulfonyl]-4*H*-1,3-benzodioxin-4-one (3). To a stirred solution of **8** (1.0 g, 4.46 mmol) in pyridine (25 mL) was added dropwise triflic anhydride (0.83 mL, 4.91 mmol) at −10 °C, and then the mixture was stirred at 0 °C for 3.5 h. After addition of ice–water, the resulting mixture was vigorously stirred, and then extracted with ether. The extracts were washed with cold dilute HCl solution, water, sat. $NaHCO_3$ solution, water, brine, dried, and concentrated. Chromatography on silica gel with hexane→hexane–EtOAc (6:1) as the eluent yielded **3** (1.50 g, 94%) as white solids: mp 58–59 °C {hexane–EtOAc (50:1)}; IR (KBr) 2993, 2954, 2850, 1747, 1624, 1431, 1284, 1203, 1138, 1061 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.74 (6H, s), 3.89 (3H, s), 6.49 (1H, d, *J*=2.3 Hz), 6.53 (1H, d, *J*=2.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.5, 56.3, 100.9, 101.1, 105.3, 106.6, 118.7 (q, *J*=320 Hz), 149.9, 157.1, 158.8, 165.5; HRMS calcd for $C_{12}H_{11}F_3O_7SNa$ $[M+Na]^+$ 379.0069, found 379.0058. Anal. Found: C, 40.64; H, 2.93. Calcd for $C_{12}H_{11}F_3O_7S$: C, 40.45; H, 3.11.

3.1.4. 1-Bromo-4,5-bis(methoxymethoxy)-2-methylbenzene (11). To a stirred solution of **10** (5.83 g, 28.7 mmol) in *N*,*N*-dimethylformamide (95 mL) was added sodium hydride (60% in mineral oil, 2.87 g, 71.8 mmol) by portions at 0 °C, and then the mixture was stirred at 0 °C for 1 h. Chloro methylmethylether (5.45 mL, 71.8 mmol) was added dropwise at 0 °C, and then the mixture was stirred at 0 °C for 6 h. After addition of sat. NH_4Cl solution, the resulting mixture was extracted with ether. The extracts were washed with water, brine, dried, and concentrated. Chromatography on silica gel with hexane–EtOAc (30:1→20:1) as the eluent yielded **11** (7.54 g, 90%) as a colorless oil: IR (neat) 2957, 2933, 2829, 1500, 1255, 1151, 1080, 1003 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.31 (3H, s), 3.51 (6H, s), 5.17 (1H, d, *J*=6.7 Hz), 5.20 (1H, d, *J*=6.7 Hz), 7.03 (1H, brs), 7.32 (1H, brs); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.2, 56.0, 95.2, 95.4, 116.1, 118.5, 120.4, 131.3, 145.5, 146.1; HRMS calcd for $C_{11}H_{15}O_4{}^{79}Br$ ($C_{11}H_{15}O_4{}^{81}Br$) $[M+H]^+$ 290.0154 (292.0134), found 290.0156 (292.0129).

3.1.5. 5-[4,5-Bis(methoxymethoxy)-2-methylphenyl]-7-methoxy-2,2-dimethyl-4*H*-1,3-benzodioxin-4-one (12). To a stirred solution of **11** (2.32 g, 7.97 mmol) in tetrahydrofuran (21 mL) was added dropwise a 1.59 M solution of *n*-butyllithium (5.5 mL, 8.77 mmol) in hexane at −78 °C, and the mixture was stirred at −78→−40 °C for 1.4 h. Then a solution of triisopropyl borate (2.02 mL, 8.77 mmol) in ether (7 mL) was added dropwise at −78 °C, and the mixture was stirred at −78 °C→rt for 1 h and at rt for 3 h. After addition of 1 M HCl solution, the resulting mixture was stirred for 45 min, and then extracted with ether. The extracts were washed with water, brine, dried, and concentrated to give **4** (1.94 g, 95%), which was employed to the next without further purification. To a stirred mixture of the above boric acid **4** (1.12 g, 4.37 mmol), **3** (1.04 g, 2.91 mmol), potassium bromide (346 mg, 2.91 mmol) and potassium phosphate (928 mg, 4.37 mmol) in dioxane (12 mL) was added tetrakis(triphenylphosphine)palladium (169 mg, 0.15 mmol), and the mixture was stirred at 100 °C for 10.5 h, cooled, and then diluted with water. The resulting mixture was extracted with EtOAc. The extracts were washed with water, brine, dried, and concentrated. Chromatography on silica gel with hexane→hexane–EtOAc (4:1→2:1) as the eluent yielded **12** (1.14 g, 93%) as white solids: mp 146–147 °C {hexane–EtOAc (20:1)}; IR (KBr) 2989, 2958, 2908, 1732, 1612, 1577, 1281, 1254, 1149, 1057 cm^{-1}; ^{1}H NMR (400 MHz,

$CDCl_3$): δ 1.73 (6H, s), 2.04 (3H, s), 3.50 (3H, s), 3.55 (3H, s), 3.85 (3H, s), 5.16 (1H, d, J=6.6 Hz), 5.22 (1H, d, J=6.7 Hz), 5.23 (1H, d, J=6.6 Hz), 5.30 (1H, d, J=6.7 Hz), 6.44 (1H, d, J=2.7 Hz), 6.45 (1H, d, J=2.7 Hz), 6.91 (1H, s), 7.02 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.4, 25.3, 26.1, 55.7, 56.2, 56.2, 95.4, 95.9, 100.5, 105.0, 105.7, 113.2, 117.0, 117.6, 129.3, 134.1, 144.8, 146.5, 146.6, 158.5, 158.9, 164.6; HRMS calcd for $C_{22}H_{26}O_8Na$ $[M+Na]^+$ 441.1519, found 441.1518. Anal. Found: C, 63.02; H, 6.51. Calcd for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26.

3.1.6. 4,4,5,5-Tetramethyl-2-(5-methyl-1,3-benzodioxol-6-yl)-1,3,2-dioxaborolane (5). To a stirred mixture of **14** (5.27 g, 20.1 mmol), bis(pinacolato)diboron (5.78 g, 22.8 mmol), and potassium acetate (5.99 g, 61.0 mmol) in *N*,*N*-dimethylformamide (150 mL) was added dichloro-[1,1′-bis(diphenylphosphino)ferrocene]palladium {$PdCl_2$-(dppf), 1.42 g, 1.94 mmol}. The mixture was stirred at 80 °C for 21 h, cooled and then diluted with EtOAc. The resulting mixture was filtered through a pad of celite, washed with brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc (9:1) as the eluent yielded **5** (4.29 g, 81%) as white solids: mp 64–65 °C; IR (KBr) 2974, 2931, 2885, 1612, 1423, 1369, 1311, 1296, 1146, 1111 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.32 (12H, s), 2.47 (3H, s), 5.90 (2H, s), 6.65 (1H, s), 7.21 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.9, 24.8, 83.2, 100.6, 110.5, 114.7, 140.4, 144.9, 149.7; HRMS calcd for $C_{14}H_{19}BO_4Na$ $[M+Na]^+$ 285.1268, found 285.1271. Anal. Found: C, 64.19; H, 7.27. Calcd for $C_{14}H_{19}BO_4$: C, 64.15; H, 7.31.

3.1.7. 7-Methoxy-2,2-dimethyl-5-(5-methyl-1,3-benzodioxol-6-yl)-4*H*-1,3-benzodioxin-4-one (15). To a stirred mixture of **3** (2.33 g, 6.54 mmol), **5** (2.23 g, 8.51 mmol), potassium carbonate (2.71 g, 19.6 mmol) in 1,2-dimethoxyethane (10 mL) was added $PdCl_2$(dppf) (240 mg, 0.328 mmol), and the mixture was stirred at 85 °C for 10 h, cooled, diluted with EtOAc, and filtered through a pad of celite. The filtrate was washed successively with water and brine, dried and concentrated. Chromatography on silica gel with hexane–EtOAc (9:1→4:1→1:1) as the eluent yielded **15** (1.50 g, 67%) white solids: mp 170–171 °C {hexane–EtOAc (5:1)}; IR (KBr) 2997, 2943, 2904, 1732, 1608, 1577, 1485, 1281, 1203, 1030 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (6H, s), 2.02 (3H, s), 3.85 (3H, s), 5.95 (1H, d, J=9.0 Hz), 5.96 (1H, d, J=9.0 Hz), 6.42 (1H, d, J=2.5 Hz), 6.45 (1H, d, J=2.5 Hz), 6.59 (1H, s), 6.70 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.6, 25.2, 26.0, 55.6, 100.5, 100.8, 105.0, 105.7, 108.5, 109.8, 113.1, 128.1, 132.9, 145.2, 146.8, 146.8, 158.5, 158.8, 164.6; HRMS calcd for $C_{19}H_{18}O_6Na$ $[M+Na]^+$ 365.0995, found 365.1001. Anal. Found: C, 66.54; H, 5.53. Calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30.

3.1.8. Altenusin (2) from 12. (i) A solution of **12** (367 mg, 0.88 mmol) in 2 M KOH–ethanol (1:1, 52 mL) was heated at 60 °C with stirring for 40 min, cooled and then diluted with water. The resulting mixture was washed with dichloromethane. The aqueous layer was acidified with cold HCl solution, and then extracted with EtOAc. The extracts were washed with brine, dried, and concentrated to give a syrup (238 mg), which was dissolved in dichloromethane–methanol (2:1, 9 mL). To this solution was added a 10% HCl solution in methanol (3 mL) and the mixture was stirred at rt for 12 h. After addition of $NaHCO_3$, the resulting mixture was filtered through a pad of celite, and concentrated. The residue was diluted with water, and acidified with cold HCl solution. The resulting mixture was extracted with EtOAc, washed with brine, dried and concentrated. The residue was treated with dichloromethane to give **2** (158 mg, 62% from **12**) as crystalline solids. The mother liquor was purified by preparative TLC {hexane–EtOAc–AcOH (25:25:1)} to give additional **2** (6 mg, 2%) as crystalline solids.

(ii) To a stirred solution of **12** (19.4 mg, 0.0464 mmol) in dichloromethane (1 mL) was added dropwise a 1.0 M solution of boron trichloride (BCl_3) in dichloromethane (0.46 mL) at 0 °C. The mixture was warmed slowly to rt and then stirred for 19.5 h. After addition of water, the resulting mixture was extracted with EtOAc. The extracts was washed with water, dried and concentrated. Chromatography on silica gel with chloroform–methanol–acetic acid (95:5:1→90:10:1) as the eluent yielded **2** (8.5 mg, 63%) as crystalline solids.

3.1.9. Altenusin (2) from 15. To a stirred solution of **15** (71.2 mg, 0.208 mmol) in dichloromethane (1 mL) was added dropwise a 1.0 M solution of BCl_3 in dichloromethane (1 mL) and then stirred for 19 h. Treatment as described above yielded **2** (48.1 mg, 80%): mp 192–194 °C (lit.[7] 194–196 °C); IR (KBr) 3309, 3012, 2974, 1651, 1612, 1577, 1520, 1254, 1207, 1157 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD): δ 1.90 (3H, s), 3.80 (3H, s), 6.16 (1H, d, J=2.6 Hz), 6.42 (1H, d, J=2.6 Hz), 6.48 (1H, s), 6.57 (1H, s); ^{13}C NMR (100 MHz, CD_3OD): δ 19.3, 55.9, 100.5, 107.0, 111.4, 116.6, 117.3, 127.3, 135.3, 143.2, 144.9, 148.0, 164.9, 165.8, 174.3; HRMS calcd for $C_{15}H_{14}O_6Na$ $[M+Na]^+$ 313.0682, found 313.0691.

3.1.10. Dehydroaltenusin (1). To a stirred solution of **2** (102 mg, 0.35 mmol) in ethanol–water (1:1, 2.2 mL) was added dropwise a 0.2 M solution of ferric chloride in water (ca. 4.0 mL) at rt. After 10 min, a yellow precipitate formed was filtered, washed with water, dried, and recrystallized from methanol–dichloromethane to **1** (62.1 mg, 61%) as yellow needles. The mother liquid collected was concentrated, subject to chromatography on silica gel {hexane–EtOAc (1:1)} and recrystallized from methanol–dichloromethane to additional **1** (20.6 mg, 21%) as yellow needles: mp 189–190 °C (lit.[5] 189–190 °C); IR (KBr) 3383, 3124, 2978, 1674, 1643, 1624, 1392, 1296, 1261, 1227, 1196, 1161, 1076 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (3H, s), 3.91 (3H, s), 6.28 (1H, s), 6.41 (1H, s), 6.63 (1H, d, J=2.4 Hz), 6.69 (1H, s), 6.73 (1H, d, J=2.4 Hz), 11.29 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.6, 56.0, 79.1, 99.8, 103.6, 104.3, 116.1, 120.7, 134.9, 145.9., 152.9, 164.5, 166.2, 167.2, 180.6; HRMS calcd for $C_{15}H_{11}O_6$ $[M-H]^-$ 287.0561, found 287.0570.

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