Total Synthesis of Altenusin and Alterlactone

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Abstract: The resorcylic lactone alterlactone, a mycotoxin produced by *alternaria sp.*, was synthesized for the first time. The total synthesis was achieved in nine steps with 69% yield starting with acetal-protected phloroglucinic acid and 6-bromopiperonal, where the longest linear sequence consists of five steps. Key step is a Suzuki coupling used for the construction of the central biaryl bond. When the final deprotection with cleavage of benzyl ethers (yielding unprotected alterlactone) was performed in a less polar solvent the biaryl mycotoxin altenusin was obtained.

Key words: polyketides, fungal metabolites, resorcylic lactones, cross-coupling, Suzuki reaction

The resorcylic lactones[1] alternariol (**1**) and alternariol 9-methyl ether (**2**) are main secondary metabolites of toxin-producing *alternaria* fungi (Figure 1).[2] Although the toxicity of these mycotoxins is low when compared with others (e.g., aflatoxins),[3] infestations with *alternaria spp.* lead to significant crop losses by fouling of tomatoes, apples, and other fruits.[4] Total syntheses have been provided for these compounds[5] and for the structurally related toxins altenuene (**3**), isoaltenuene (**4**),[6] and neoaltenuene (**5**).[7]

Minor toxins produced by *alternaria spp.*[8] and other fungi[9] are altenusin (**6**) and its oxidized derivative dehydroaltenusin (**7**). Altenusin is a selective pp60c-Src kinase inhibitor (IC_{50} = 20 nM, 400 × more active than against other tested protein kinases),[10] a moderate inhibitor of myosin light-chain kinase (IC_{50} = 340 μM),[11] and a specific inhibitor of neutral sphingomyelinase (K_i = 20 μM),[12] inhibits trypanothione reductase from the parasite *Trypanosoma cruzi* (IC_{50} = 4.3 μM)[13] and shows a broad antimicrobial activity against several multidrug-resistant bacterial and fungal strains,[14] but is only moderately active against HIV-1 integrase.[9b] It had originally been synthesized en route to dehydroaltenusin (**7**) in eight steps with 27% yield starting with commercially available phloroglucinic acid and 4-methylcatechol, where the longest linear sequence consisted of five steps.[15]

Alterlactone (**8**) was isolated from a fungal endophyte *alternaria sp.* It shows moderate cytotoxic in vitro activity against L5178Y cells (11.8% growth at 10 μmol/mL).[16] Further biological activities have not been reported for **8**, nor has a total synthesis been published for this natural product. To supply sufficient amounts of material for further testings we aimed for a convergent total synthesis of several resorcylic lactones. Herein we report on the total synthesis of altenusin (**6**) and of alterlactone (**8**).

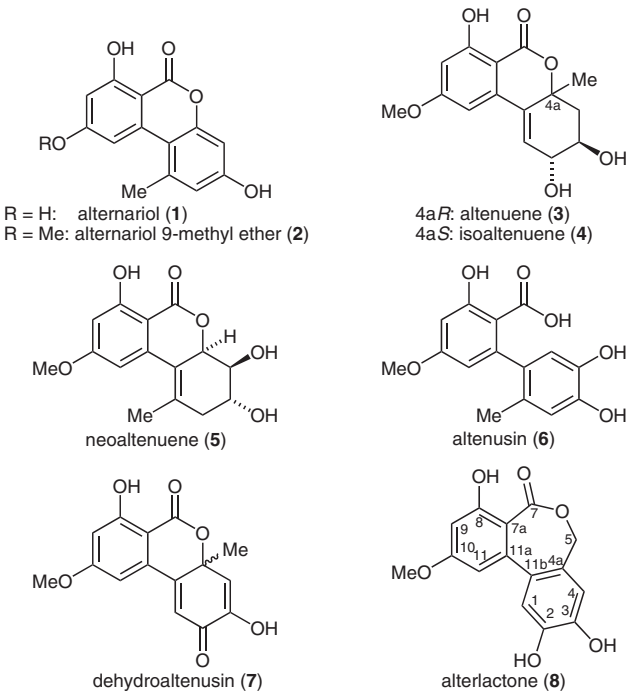
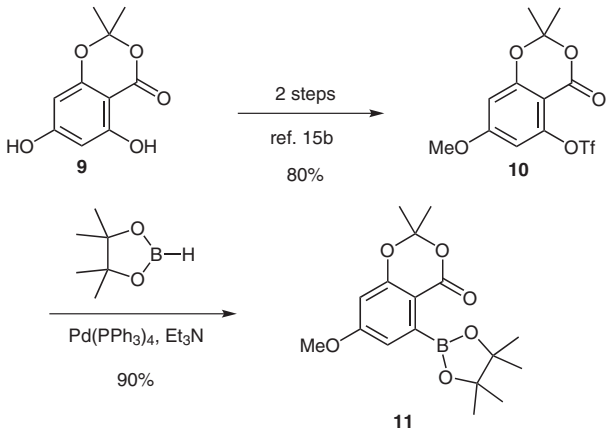


Figure 1 Selection of toxins produced by *alternaria spp.*



Scheme 1 Synthesis of boronate **11**

The projected strategy for a convergent synthesis of altenusin (**6**) and alterlactone (**8**) resembles a protocol, which had already been successfully applied to the total synthesis of other resorcylic lactones. Key reaction would be a Suzuki reaction of a suitably substituted aryl boronate **11**

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with a highly oxygenated aryl bromide. Starting with commercially available acetal-protected phloroglucinic acid **9** a pinacol-derived boronate **11** was obtained with 72% yield (Scheme 1).[15b,17,18]

While bromination of *O*-methylvanilline (**12**, Figure 2) was successful,[19] the required complete demethylation of the resulting carbaldehyde **13** to yield **15** was not possible with any of the methods investigated.[20]

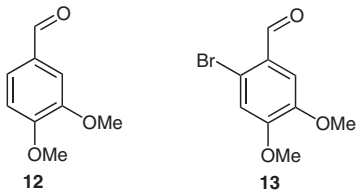
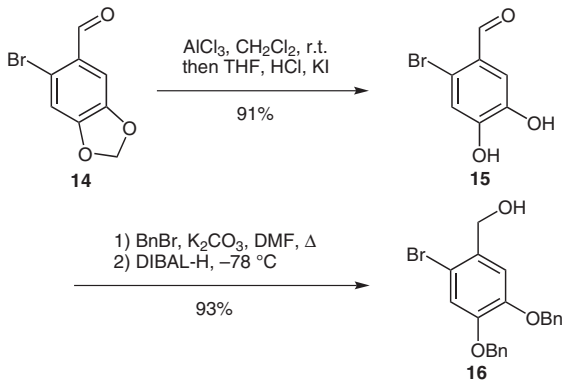


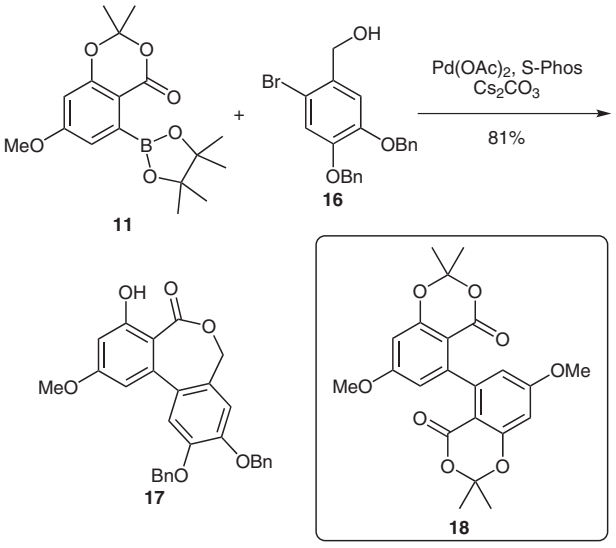
Figure 2

We therefore used commercially available bromopiperonal (**14**) as starting material, which was successfully deprotected with anhydrous aluminum chloride followed by refluxing with hydrochloric acid and potassium iodide in order to cleave the intermediately formed chloromethoxybenzaldehyde.[20a] Double benzylation using a modified protocol of Reitz et al.[20a] and reduction of the aldehyde function with diisobutylaluminum hydride yielding the unknown alcohol **16** (Scheme 2) turned out to afford better results than a previously published method.[20b,21]



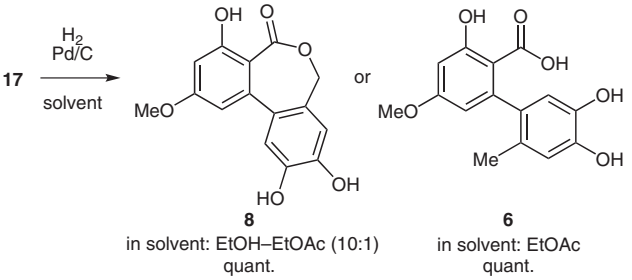
Scheme 2 Synthesis of brominated derivative **16**

Suzuki coupling with concomitant translactonization was achieved with palladium(II) acetate as catalyst, cesium carbonate as base, and S-Phos as ligand in a 81% yield (Scheme 3).[22] Minor amounts of the homocoupling product **18** (ca. 26% calculated on the boronate **11**, which was used in excess) were obtained with this protocol, as already reported for similar couplings.[18] Though formation of this side product could not be prevented, it was easily separated by conventional chromatography. Since traces of the starting material **16** could not be removed even by medium-pressure liquid chromatography (MLPC) purification with HPLC turned out to be necessary. This ultimately afforded the coupling product **17** in high yield.



Scheme 3 Suzuki coupling yielding protected alterlactone **17**

The reaction outcome in the hydrogenolysis of benzyl-protected alterlactone **17** was strongly dependent from the solvent used (Scheme 4). Hydrogenolysis in the presence of palladium on charcoal quantitatively led to alterlactone (**8**) when a mixture of ethanol and ethyl acetate (10:1) was used as solvent, while utilization of ethyl acetate as the sole solvent furthermore led to further reduction (cleavage of the benzylic C–O bond in the seven-membered ring) with formation of altenusin (**6**).



Scheme 4 Hydrogenolysis of benzyl-protected alterlactone **17**

This first total synthesis of alterlactone (**8**) was achieved in 69% with nine steps starting with acetal-protected phloroglucinic acid **9** and 6-bromopiperonal (**14**), where the longest linear sequence consists of five steps. Similarly, a competitive further total synthesis of altenusin (**6**) was achieved with the same yield and in the same number of steps. Since dehydroaltenusin had previously been synthesized from altenusin (**6**),[15] this work is furthermore a formal synthesis of dehydroaltenusin (**7**). NMR spectroscopic data of synthesized alterlactone (**8**) are in full agreement with published data of the natural product,[16] giving unambiguous evidence that the proposed structure of alterlactone (**8**) is correct (Table 1).

Table 1 Comparison of NMR Data for Alterlactone (**8**)[16]

Position[a]	^{1}H NMR data		^{13}C NMR data	
	Natural	Synthesized	Natural	Synthesized
1	7.03 (s)	7.04 (s)	115.5	115.5
2			146.6	146.6
3			145.9	145.9
4	6.90 (s)	6.91 (s)	115.5	115.5
4a			140.0	140.1
5	4.80 (d, *J* = 11.0 Hz), 4.85 (d, *J* = 11.3 Hz)	4.83–4.85	67.8	67.8
7			168.7	168.8
7a			109.5	109.5
8			159.9	160.0
9			100.8	100.8
10	6.45 (d, *J* = 2.2 Hz)	6.46 (*J* = 2.5 Hz)	162.2	162.2
11			105.0	105.0
11a	6.50 (d, *J* = 2.2 Hz)	6.51 (*J* = 2.4 Hz)	126.6	126.6
11b			129.8	129.9
OMe	3.81 (s)	3.82	55.4	55.4
2-OH, 3 OH	9.37, 9.47 (br s)	9.38, 9.47 (br s)		
8-OH	10.21 (br s)	10.21 (br s)		

[a] The numbering is given in Figure 1. A different numbering had been given in the original literature.[16]

9,10-Bis(benzyloxy)-4-hydroxy-2-methoxydibenzo[*c*,*e*]oxepin-5(7*H*)-one (17)

Arylbromide **16** (210 mg, 0.526 mmol), boronate **11** (228 mg, 684 μmol), Cs_2CO_3 (0.514 g, 1.58 mmol), $Pd(OAc)_2$ (3.5 mg, 16 μmol), and S-Phos (purity 97%, 13.0 mg, 32.0 μmol) were dissolved under an Ar atmosphere in degassed dioxane–H_2O (7:1, 10 mL), and the mixture was stirred for 20 h at 80 °C (monitoring with TLC) and cooled. A sat. NH_4Cl solution (10 mL) was added, and the mixture was extracted with EtOAc (2 × 25 mL). The organic layers were dried (Na_2SO_4) and concentrated, and the residue was purified by chromatography (silica gel, cyclohexane–EtOAc = 10:1) to yield side product **18** (37 mg) and a mixture (240 mg) of coupling product **17** and bromide **16**. Aliquots of this mixture (150 mg) were purified with preparative HPLC (MeCN–H_2O = 75:25 with 0.1% TFA to 98:2 with 0.1% TFA) to yield **17** as a colorless solid (119 mg, 0.254 mmol, 81%); mp 150–154 °C; R_f = 0.54 (hexanes–EtOAc = 2:1). IR (KBr): 2915, 1641, 1566, 1514, 1422, 1346, 1252, 1200, 1150, 1075, 1044, 987, 967, 916, 835, 753 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.85 (s, 3 H, OMe), 4.79–4.81 (m, 1 H, CH_2), 4.98–5.02 (m, 1 H, CH_2), 5.23 (s, 4 H, Bn), 6.41 (d, ^4J = 2.6 Hz, 1 H, ArH), 6.55 (d, ^4J = 2.6 Hz, 1 H, ArH), 6.96 (s, 1 H, ArH), 7.09 (s, 1 H, ArH), 7.47–7.32 (m, 10 H, Bn), 10.62 (br s, 1 H, OH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 29.7 (CH_3), 55.6 (CH_3), 71.3 (CH_2), 71.4 (CH_2), 71.5 (CH_2), 100.9 (CH), 106.3 (C),108.5 (CH), 113.7 (CH), 114.1 (CH), 116.0 (CH), 127.2 (C), 127.2 (CH), 127.4 (CH), 127.5 (C), 128.1 (CH), 128.1 (CH), 128.6 (CH), 128.7 (CH), 128.8 (CH), 132.6 (C), 136.6 (C), 136.7 (C), 141.0 (C), 149.4 (C), 149.9 (C), 163.8 (C), 163.9 (C), 172.8 (C) ppm. MS–FAB: *m/z* = 469 $[M + H]^+$. HRMS–FAB: *m/z* calcd for $C_{29}H_{25}O_6$ 469.1651 $[MH^+]$; found: 469.1649.

Side Product 18: 7,7′-Dimethoxy-2,2,2′,2′-tetramethyl-4,4′-dioxo-4*H*,4′*H*-[5,5′]bi{benzo[*d*][1,3]dioxinyl}

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.73 (s, 6 H, CH_3), 1.76 (s, 6 H, CH_3), 3.85 (s, 6 H, OMe), 6.46 (d, 2 H, ^4J = 2.5 Hz, ArH), 6.48 (d, 2 H, ^4J = 2.5 Hz, ArH).

4,9,10-Trihydroxy-2-methoxydibenzo[*c*,*e*]oxepin-5(7*H*)-one [Alterlactone (8)]

Pd/C (10%, 16.0 mg, 154 μmol) was added to a solution of protected alterlactone **17** (18.0 mg, 38.0 μmol) in EtOAc (0.05 mL) and EtOH (0.5 mL). The atmosphere was replaced with H_2, and the mixture was stirred for 5 h at r.t. (monitoring with TLC). The mixture was filtered, and the filtrate was concentrated to yield alterlactone (**8**) as a colorless solid (10.9 mg, 38.0 μmol, quant.). R_f = 0.29 (CH_2Cl_2–MeOH = 10:1). IR (KBr): 3080, 1647, 1604, 1566, 1525, 1458, 1421, 1370, 1268, 1201, 1152, 1112, 1075, 1022, 990, 824, 773, 621 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.82 (s, 3 H, OMe), 4.83–4.85 (m, 2 H, CH_2), 6.46 (d, ^4J = 2.5 Hz, 1 H, ArH), 6.51 (d, ^4J = 2.4 Hz, 1 H, ArH), 6.91 (s, 1 H, ArH), 7.04 (s, 1 H, ArH), 9.38 (br s, 1 H, OH), 9.47 (br s, 1 H, OH), 10.21 (br s, 1 H, OH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 55.4 (CH_3, OCH_3), 67.8 (CH_2, C-5), 100.8 (C, C-9), 105.0 (CH, C-11), 109.5 (C, C-7a), 115.5 (2 CH, C-4, C-1), 126.6 (C, C-11a), 129.9 (C, C-11b), 140.1 (C, C-4a), 145.9 (C, C-3), 146.6 (C, C-2), 150.0 (C, C-8), 162.2 (C, C-10), 168.8 (C, C-7) ppm; MS–FAB: *m/z* = 289 $[M + H]^+$. HRMS–FAB: *m/z* calcd for $C_{15}H_{13}O_6$: 289.0712 $[MH^+]$; found: 289.0709. The analytical data are in full accordance with published data.[16]

3,4′,5′-Trihydroxy-5-methoxy-2′-methyl-biphenyl-2-carboxylic Acid [Altenusin (6)]

Pd/C (10%, 18.0 mg, 171 μmol) was added to a solution of protected alterlactone **17** (20.0 mg, 43.0 mol) in EtOAc (1 mL). The atmosphere was replaced with H_2, and the mixture was stirred for 8 h at r.t. (monitoring with TLC). The mixture was filtered, and the filtrate was concentrated to yield altenusin (**6**) as a colorless solid (11.9 g, 43.0 μmol, quant.). R_f = 0.13 (CH_2Cl_2–MeOH = 10:1). ^{1}H NMR (250 MHz, CD_3OD): δ = 1.92 (s, 3 H, CH_3), 3.81 (s, 3 H, OMe), 6.17 (d, ^4J = 2.6 Hz, 1 H, ArH), 6.43 (d, ^4J = 2.6 Hz, 1 H, ArH), 6.49 (s, 1 H, ArH), 6.59 (s, 1 H, s, ArH) ppm. The analytical data are in full accordance with published data.[9b,15b]

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett. Included are experimental and spectroscopic data for all compounds, and spectra of title compounds.

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