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Synthesis of novel antifungal phthalides produced by a wheat rhizosphere fungus

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ABSTRACT

Two antifungal phthalides produced by a wheat rhizosphere fungus have been synthesized using the Alder–Rickert reaction to construct their common isobenzofuranone core structure. The absolute configuration of one of the two phthalides has been determined to be *S* by synthesizing its (*S*)- and (*R*)-enantiomer and comparing their optical rotations with that of the natural product.

1. Introduction

Take-all, one of the most serious diseases of wheat, is caused by infection with the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici*.[1] This disease, which damages the roots of wheat, occurs in continuously cropped wheat fields, causing massive reductions in wheat production.[2] However, the disease does not occur in some wheat fields, even when they are cropped continuously over long periods. Narita and Suzuki isolated an unidentified fungus from the healthy roots of wheat grown in a continuously cropped field, and provisionally named it 'Sterile Dark.'[3] They found that when Sterile Dark was applied to wheat seeds, it colonized the roots and suppressed the growth of the pathogenic take-all fungus on the roots.[3] This finding led Takahashi and co-workers to assume that some antifungal substance was produced by Sterile Dark that inhibited the growth of the take-all fungus. Based on this assumption, they screened the culture broth of Sterile Dark for antifungal substances directed against the take-all fungus and isolated two novel phthalides, **1** and **2** (Fig. 1).[4] Compound **1** exhibited antifungal activity against *G. graminis* var. *tritici* and *Cladosporium herbarum*, whereas compound **2** had activity only against *C. herbarum*.[4] The agriculturally important biological activities of **1** and **2**, and the undefined absolute configuration of **2**, prompted us to synthesize these phthalides. We herein describe the first synthesis of **1** and both enantiomers of **2**, which allowed us to unambiguously determine the absolute configuration of the natural enantiomer of **2** as *S*.

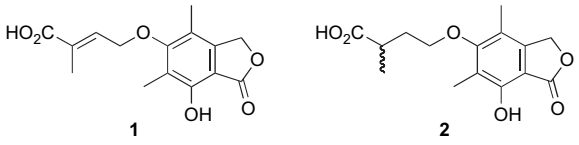

Figure 1. Antifungal phthalides produced by Sterile Dark.

2. Results and discussion

Our retrosynthetic analysis of **1** and the enantiomers of **2** is shown in Scheme 1. The target molecules, **1** and **2**, would be obtainable by O-alkylation of hydroxy phthalide **A** with halides **B** and **C**, respectively. To construct the tetra-substituted phthalide core **A**, we planned to utilize the Alder–Rickert reaction of cyclohexadiene **E** with dimethyl acetylenedicarboxylate (DMAD) to form aromatic diester **D**. Selective reduction of the ester functionality *meta* to both of the two alkoxy groups of **D** would afford the phthalide core **A**. The alkylating agent **B** (X=Br, R^2=Me) is a known compound, and both enantiomers of **C** would readily be obtained by Evans' asymmetric alkylation using chiral oxazolidinone auxiliaries.

The synthesis of the key intermediate **A** (**10** in Scheme 2) began with a two-step preparation of **5** from 2-methyl-1,3-cyclohexanedione (**4**) according to procedures in the literature.[5–7] The enone **5** was converted into unstable TES-enol ether **6**,[8] which was then subjected without purification to the Alder–Rickert reaction with

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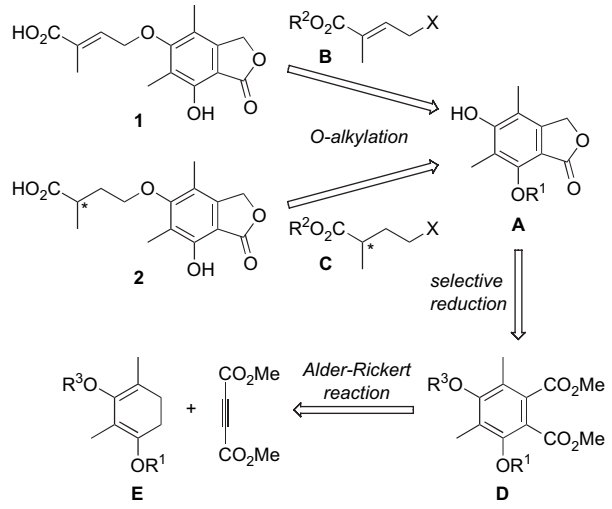
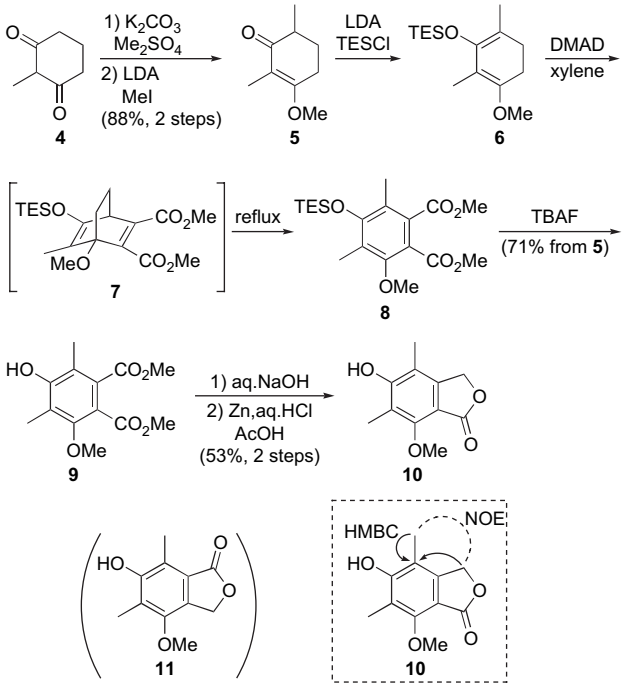


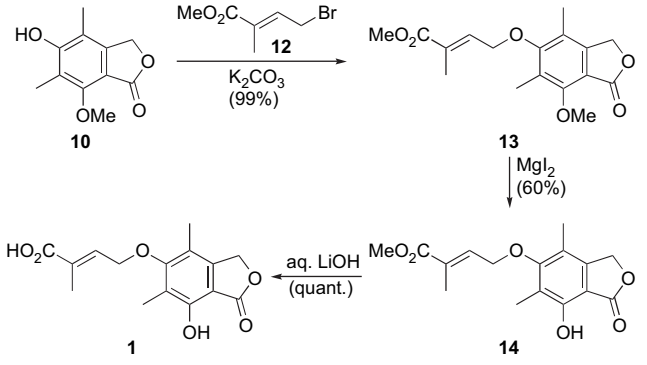
Scheme 1. Retrosynthetic analysis of **1** and **2**.



Scheme 2. Preparation of phthalide core **10**.

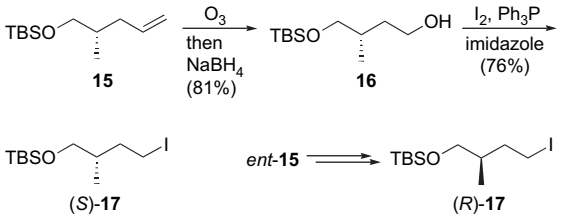
DMDA in *m*-xylene.[9,10] The cycloaddition of DMDA to **6** to form bicyclic intermediate **7** and subsequent elimination of ethylene from the adduct proceeded smoothly to yield aromatic diester **8**. The TES-protected diester **8**, obtained unfortunately as an inseparable mixture with DMDA employed in excess, was treated with TBAF to produce, after chromatographic purification, phenolic diester **9** in 71% overall yield from **5**. Selective reduction of the ester group *meta* to both the hydroxy and methoxy functionalities of **9** was achieved in two steps according to Patterson's protocol:[9,11] (1) hydrolysis of the diester to the corresponding dicarboxylic acid intermediate; and (2) reductive treatment of the intermediate with Zn in 12 M HCl/AcOH to form lactone **10** via an acid anhydride intermediate, which is in equilibrium with the dicarboxylic acid under these reaction conditions.[9] Fortunately, this reduction proceeded highly regioselectively to produce **10** and its isomer **11** in a ratio of >20:1, and the undesired minor isomer **11** was readily removed by SiO_2 column chromatography. The structure of **10** was confirmed by observing the NOE and HMBC correlations, as shown in Scheme 2.[12]

The phthalide core **10** was treated with bromo ester **12**[13] in acetone in the presence of K_2CO_3 to give alkylation product **13**, which was then exposed to MgI_2 in ether/toluene to deprotect the methoxy moiety (Scheme 3).[14,15] The demethylation product **14**, obtained in a moderate yield of 60%, was hydrolyzed with aqueous LiOH in MeOH to produce the antifungal phthalide **1** in quantitative yield. The ^1H and ^{13}C NMR spectra of the synthetic material were identical with those of the natural product.



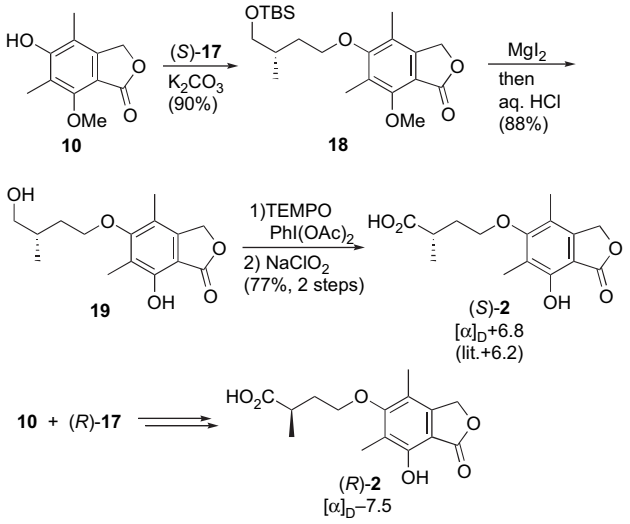
Scheme 3. Synthesis of **1**.

Having completed the synthesis of the achiral phthalide **1**, we next turned our attention to the synthesis of both enantiomers of **2**, which contain one stereogenic center. *O*-Protected iodides (*S*)-**17** and (*R*)-**17** to alkylate the phthalide core **10** were prepared by the reaction sequence shown in Scheme 4. Known olefinic compound **15**, prepared via Evans' asymmetric alkylation protocol,[16] was subjected to ozonolysis conditions to give alcohol **16** after reductive workup with $NaBH_4$. The alcohol **16** was then converted into the corresponding iodide (*S*)-**17** by treatment with I_2, Ph_3P, and imidazole in THF. The (*R*)-enantiomer of **17** [(*R*)-**17**] was obtained from *ent*-**15** by the same two-step sequence of reactions.[16,17]



Scheme 4. Preparation of both enantiomers of alkylating agent **17**.

With both enantiomers of **17** in hand, we moved on to the final stage of the synthesis of (*S*)- and (*R*)-**2** (Scheme 5). O-Alkylation of **10** with (*S*)-**17** in acetone in the presence of K_2CO_3 proceeded cleanly to give **18**. Deprotections of the methyl- and TBS-protected hydroxy groups of **18** to form **19** were conducted in a single operation by treatment of **18** with MgI_2 followed by exposure of the resulting demethylated intermediate to aq HCl to remove the TBS group. Oxidation of alcohol **19** into carboxylic acid (*S*)-**2** was performed in two steps by first converting **19** into an aldehyde intermediate with TEMPO/PhI(OAc)$_2$[18] and then subjecting the aldehyde to Pinnick oxidation conditions.[19–21] By the same four-step sequence of reactions, (*R*)-**17** was transformed into (*R*)-**2**. The ^1H and ^{13}C NMR spectra of (*S*)-**2** and (*R*)-**2** were identical with those of the natural product, which allowed us to confirm the proposed gross structure of the antifungal phthalide **2**. Comparison of the specific rotations of (*S*)-**2** ($[\alpha]_D^{22}$ +6.8) and (*R*)-**2** ($[\alpha]_D^{22}$ −7.5) with that of the natural compound ($[\alpha]_D$ +6.2)[4] clearly showed the absolute configuration of the chiral phthalide to be *S*.



Scheme 5. Synthesis of both enantiomers of **2**.

3. Conclusion

The synthesis of antifungal substance **1** was achieved in 20% overall yield from **4** via the alkylation of phthalide intermediate **10** with allylic bromide **12**; the intermediate **10** in turn was prepared via the Alder–Rickert reaction between cyclohexadiene derivative **6** and DMAD. Alkylation of **10** with (*S*)- and (*R*)-**17**, prepared using Evans' asymmetric alkylation methodology, and subsequent deprotection and oxidation afforded both enantiomers of chiral phthalide **2** in 20% overall yield from **4**. Comparison of the specific rotations of (*S*)- and (*R*)-**2** with that of the natural product led to the unambiguous conclusion that the absolute configuration of the natural phthalide (**2**) is *S*.

4. Experimental

4.1. General

IR spectra were recorded by a Jasco FR/IR-4100 spectrometer. NMR spectra were recorded with TMS as an internal standard in $CDCl_3$ by a Varian Gemini 2000 spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C), a Varian UNITY plus-500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C), or a Varian UNITY plus-600 spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C) unless otherwise stated. Optical rotation values were measured with a Jasco DIP-371 polarimeter, and mass spectra were obtained with a Jeol JMS-700 spectrometer. Merck silica gel 60 (70–230 mesh) was used for silica gel column chromatography.

*4.1.1. 3-Methoxy-2,6-dimethyl-2-cyclohexen-1-one (**5**)*

To a stirred solution of **4** (10.0 g, 79.3 mmol) in acetone (80 ml) were successively added K_2CO_3 (12.1 g, 87.2 mmol) and Me_2SO_4 (8.27 ml, 87.2 mmol) at room temperature. After being stirred overnight at reflux, the mixture was filtered, and the filtrate was concentrated in vacuo to give crude 3-methoxy-2-methyl-2-cyclohexen-1-one. The enone was dissolved in THF (20 ml) and added dropwise to a stirred solution of LDA [prepared by treating a solution of diisopropylamine (12.6 ml, 89.9 mmol) in THF (60 ml) with *n*-BuLi (1.6 M in hexane, 56.2 ml, 89.9 mmol) at −10 °C] at −78 °C. After 30 min, MeI (4.94 ml, 79.3 mmol) and HMPA (15.2 ml, 87.4 mmol) were added. The reaction mixture was gradually warmed to room temperature and stirred overnight before being quenched with satd NH_4Cl aq and extracted with EtOAc. The extract was successively washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/EtOAc=10:1) to give 10.8 g (88%) of **5** as a colorless solid, recrystallization of which from hexane afforded colorless prisms: mp 46.0–46.5 °C; IR ν 2932 (m), 1616 (vs), 1377 (s), 1355 (s), 1247 (s), 1105 (s); ^1H NMR (300 MHz) δ 1.14 (3H, d, J=6.9 Hz), 1.64–1.74 (1H, m), 1.69 (3H, t, J=1.6 Hz), 2.08 (1H, dq, J=13.2, 4.5 Hz), 2.27 (1H, ddq, J=11.3, 4.5, 6.9 Hz), 2.46–2.59 (1H, m), 2.59–2.70 (1H, m), 3.81 (3H, s); ^{13}C NMR (75 MHz) δ 7.4, 15.5, 23.8, 28.6, 39.2, 54.9, 114.0, 170.6, 201.2; HRMS (FAB) m/z calcd for $C_9H_{15}O_2$ ($[M+H]^+$) 155.1072, found 155.1080.

*4.1.2. Dimethyl 4-hydroxy-6-methoxy-3,5-dimethyl-1,2-benzenedicarboxylate (**9**)*

To a stirred solution of LDA, prepared by treating a solution of diisopropylamine (3.00 ml, 21.4 mmol) in THF (20 ml) with *n*-BuLi (1.6 M in hexane, 13.4 ml, 21.4 mmol) at −10 °C, were successively added TESCl (2.74 ml, 21.4 mmol) and a solution of **5** (3.00 g, 19.5 mmol) in THF (10 ml) at −78 °C. After 1 h, the reaction mixture was quenched with ice-cold satd $NaHCO_3$ aq and extracted with hexane. The extract was washed with brine, dried (K_2CO_3), and concentrated in vacuo to give **6**, which was then dissolved in *m*-xylene (20 ml). To the solution was added DMAD (4.78 ml, 38.9 mmol) at −50 °C while stirring and the mixture was gradually warmed to 70 °C. After being stirred for 4 h at 70 °C, the mixture was refluxed for 4 h and then concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/EtOAc=5:1) to give an inseparable mixture of **8** and DMAD. The mixture was dissolved in THF (20 ml) and cooled to 0 °C. To the stirred solution was added dropwise a solution of TBAF (1.0 M in THF, 29.2 ml, 0.292 mmol). After being stirred overnight at room temperature, the mixture was quenched with satd NH_4Cl aq and extracted with EtOAc. The extract was successively washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/EtOAc=3:1) to give 3.68 g (71%) of **9** as a white solid, recrystallization of which from H_2O/MeOH afforded colorless needles: mp 118.0–118.5 °C; IR ν 3450 (br m), 1713 (vs), 1303 (m), 1197 (s), 1113 (m); ^1H NMR (500 MHz) δ 2.21 (3H, s), 2.23 (3H, s), 3.79 (3H, s), 3.86 (3H, s), 3.87 (3H, s), 5.08 (1H, br s, OH); ^{13}C NMR (125 MHz) δ 9.1, 12.6, 52.40, 52.42, 62.2, 118.0, 118.3, 119.8, 131.7, 155.6, 155.7, 167.1, 168.5; HRMS (FAB) m/z calcd for $C_{13}H_{17}O_6$ ($[M+H]^+$) 269.1025, found 269.1024.

*4.1.3. 5-Hydroxy-7-methoxy-4,6-dimethyl-1(3H)-isobenzofuranone (**10**)*

To a stirred solution of **9** (0.580 g, 2.16 mmol) in MeOH (4.3 ml) was added a solution of NaOH (0.520 g, 13.0 mmol) in H_2O (7 ml) at room temperature, and the reaction mixture was warmed to 50 °C. After 6 h, the mixture was cooled to room temperature, diluted with water, and extracted with Et_2O. The aqueous layer was acidified with 6 M HCl aq and extracted with EtOAc. The EtOAc solution was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was diluted with a mixture of 12 M HCl aq and AcOH (1:4, 3.3 ml), and the mixture was warmed to 70 °C with stirring. The mixture was treated with Zn dust for 6 h (1 g/h, 6 g in total), before being quenched with water and extracted with EtOAc. The extract was successively washed with satd $NaHCO_3$ aq and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 ($CHCl_3$/MeOH=50:1) to give 241 mg (54%) of **10** as a white solid, recrystallization of which from H_2O/EtOH afforded colorless needles: mp 207.0–208.0 °C; IR ν 3230 (br m), 1715 (s), 1592 (m), 1487 (m), 1321 (m); ^1H NMR (300 MHz) δ 2.14 (3H, s), 2.21 (3H, s), 4.04 (3H, s), 5.14 (2H, s), 5.24 (1H, br s, OH); ^{13}C NMR (150 MHz, acetone-d_6) δ 9.1, 11.2, 62.0, 68.6, 109.1, 113.6, 118.6, 147.7, 156.9, 160.3, 169.4; HRMS (FAB) m/z calcd for $C_{11}H_{13}O_4$ ($[M+H]^+$) 209.0814, found 209.0816.

4.1.4. Methyl (E)-4-[(1,3-dihydro-7-methoxy-4,6-dimethyl-1-oxo-5-isobenzofuranyl)oxy]-2-methyl-2-butenoate (**13**)

To a stirred mixture of **10** (14.0 mg, 67.2 μmol) and K_2CO_3 (20.8 mg, 0.154 mmol) in acetone (2 ml) was added dropwise **12** (47.1 mg, 0.244 mmol) at room temperature. The mixture was refluxed for 1 h, and then concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/EtOAc=5:1) to give 21.4 mg (99%) of **13** as a white solid, a portion of which was recrystallized from $CHCl_3$/MeOH to afford colorless prisms: mp 109.0–109.5 °C; IR ν 1758 (vs), 1715 (vs), 1250 (m), 1131 (m), 1105 (m); ^{1}H NMR (500 MHz) δ 1.87 (3H, d, J=1.0 Hz), 2.18 (3H, s), 2.25 (3H, s), 3.80 (3H, s), 4.05 (3H, s), 4.54 (2H, d, J=6.0 Hz), 5.14 (2H, s), 7.03 (1H, tq, J=6.0, 1.0 Hz); ^{13}C NMR (125 MHz) δ 9.6, 11.6, 12.9, 52.1, 62.2, 68.2, 69.5, 112.7, 120.0, 125.3, 129.9, 135.9, 146.1, 156.6, 161.4, 167.6, 168.3; HRMS (FAB) m/z calcd for $C_{17}H_{21}O_6$ ([M+H]$^+$) 321.1338, found 321.1347.

4.1.5. Methyl (E)-4-[(1,3-dihydro-7-hydroxy-4,6-dimethyl-1-oxo-5-isobenzofuranyl)oxy]-2-methyl-2-butenoate (**14**)

To a stirred suspension of MgI_2 in Et_2O/toluene, prepared from Mg (6.5 mg, 0.27 mmol) and I_2 (68 mg, 0.27 mmol) in Et_2O/toluene (1:2, 0.8 ml), was added a solution of **13** (72.1 mg, 0.225 mmol) in Et_2O/toluene (1:2, 3 ml) at room temperature. The mixture was refluxed for 2.5 h, and then quenched with ice-cold 1 M HCl aq. The mixture was extracted with EtOAc and the extract was successively washed with satd $NaHCO_3$ aq and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/EtOAc=5:1) to give 41.3 mg (60%) of **14** as a white solid, recrystallization of which from H_2O/MeOH afforded colorless needles: mp 142.0–143.0 °C; IR ν 3419 (m), 1719 (s), 1627 (m), 1316 (m), 1274 (m), 1154 (m), 1074 (m); ^{1}H NMR (300 MHz) δ 1.87 (3H, s), 2.15 (3H, s), 2.21 (3H, s), 3.80 (3H, s), 4.55 (2H, d, J=6.0 Hz), 5.21 (2H, s), 7.03 (1H, dq, J=6.0, 1.2 Hz), 7.70 (1H, br s, OH); ^{13}C NMR (75 MHz) δ 8.7, 11.5, 12.8, 52.0, 69.6, 70.0, 106.4, 116.6, 118.6, 130.0, 136.0, 143.6, 153.9, 162.5, 167.8, 173.0; HRMS (FAB) m/z calcd for $C_{16}H_{19}O_6$ ([M+H]$^+$) 307.1182, found 307.1185.

4.1.6. (E)-4-[(1,3-Dihydro-7-hydroxy-4,6-dimethyl-1-oxo-5-isobenzofuranyl)oxy]-2-methyl-2-butenoic acid (**1**)

To a stirred solution of **14** (28.5 mg, 93.0 μmol) in a mixture of water and MeOH (1:2, 3 ml) was added LiOH·H_2O (16.6 mg, 0.396 mmol) at room temperature. After 6 h, the mixture was extracted with Et_2O, and the aqueous layer was acidified with 6 M HCl aq. The aqueous layer was extracted with EtOAc, and the extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give 26.9 mg (99%) of **1** as a white solid, recrystallization of which from H_2O/MeOH afforded colorless needles: mp 180.5–181.5 °C; IR ν 3433 (m), 1729 (s), 1685 (s), 1261 (m), 1146 (s), 1072 (m); ^{1}H NMR (500 MHz) δ 1.88 (3H, s), 2.16 (3H, s), 2.22 (3H, s), 4.58 (2H, d, J=5.9 Hz), 5.22 (2H, s), 7.16 (1H, t, J=5.9 Hz), 7.72 (1H, br s, OH); ^{13}C NMR (75 MHz) δ 8.8, 11.6, 12.5, 69.6, 70.0, 106.5, 116.6, 118.7, 129.3, 138.5, 143.6, 154.0, 162.5, 171.9, 173.1; HRMS (FAB) m/z calcd for $C_{15}H_{17}O_6$ ([M+H]$^+$) 293.1025, found 293.1027.

4.1.7. (S)-4-tert-Butyldimethylsilyloxy-3-methyl-1-butanol (**16**)

Ozone was bubbled into a stirred solution of **15** (0.156 g, 0.728 mmol; $[\alpha]_D^{24}$ −1.1 (*c* 1.01, $CHCl_3$), lit.[16] $[\alpha]_D^{20}$ −1.0 (*c* 1.0, $CHCl_3$)) in MeOH (5 ml) for 10 min at −78 °C. After the addition of $NaBH_4$ (0.138 g, 3.65 mmol), the mixture was stirred for 30 min at −78 °C, and then quenched with satd NH_4Cl aq. The mixture was extracted with EtOAc, and the extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/EtOAc=5:1) to give 0.129 g (81%) of **16** as a colorless oil. $[\alpha]_D^{27}$ −6.73 (*c* 6.50, $CHCl_3$), lit.[22] $[\alpha]_D^{24}$ −6.96 (*c* 4.80, $CHCl_3$); IR ν 3365 (br m), 1254 (m), 1092 (s), 835 (s); ^{1}H NMR (300 MHz) δ 0.07 (6H, s), 0.90 (3H, d, J=6.9 Hz), 0.91 (9H, s), 1.49–1.67 (2H, m), 1.71–1.84 (1H, m), 2.81 (1H, br s, OH), 3.43 (1H, dd, J=9.9, 7.1 Hz), 3.54 (1H, dd, J=9.9, 4.7 Hz), 3.63 (1H, ddd, J=11.0, 7.1, 5.5 Hz), 3.72 (1H, dt, J=11.0, 5.6 Hz); ^{13}C NMR (75 MHz) δ −5.7, −5.6, 17.2, 18.2, 25.8 (3C), 33.8, 37.9, 61.1, 68.7; HRMS (FAB) m/z calcd for $C_{11}H_{27}O_2Si$ ([M+H]$^+$) 219.1780, found 219.1783.

4.1.8. (S)-1-tert-Butyldimethylsiloxy-4-iodo-2-methylbutane [(S)-**17**]

To a stirred solution of **16** (128 mg, 0.586 mmol) and Ph_3P (0.385 g, 1.47 mmol) in THF (2 ml) were successively added imidazole (99.8 mg, 1.47 mmol) and I_2 (297 mg, 1.17 mmol) at 0 °C in the dark. The mixture was warmed to room temperature, stirred for 3 h, and quenched with satd $Na_2S_2O_3$ aq. The mixture was extracted with EtOAc, and the extract was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane) to give 147 mg (76%) of (*S*)-**17** as a colorless oil. $[\alpha]_D^{26}$ −10.5 (*c* 2.35, $CHCl_3$), lit.[22] $[\alpha]_D^{27}$ −10.5 (*c* 2.35, $CHCl_3$); IR ν 1256 (m), 1094 (m), 838 (m); ^{1}H NMR (500 MHz) δ 0.04 (6H, s), 0.88 (3H d, J=6.5 Hz), 0.89 (9H, s), 1.61–1.69 (1H, m), 1.70–1.79 (1H, m), 1.97–2.05 (1H, m), 3.21 (1H, dt, J=9.5, 7.5 Hz), 3.28 (1H, ddd, J=9.5, 8.0, 6.0 Hz), 3.43 (1H, dd, J=10.0, 6.0 Hz), 3.47 (1H, dd, J=10.0, 6.0 Hz); ^{13}C NMR (125 MHz) δ −5.43, −5.42, 5.3, 15.9, 18.3, 25.9 (3C), 36.6, 37.6, 67.3; HRMS (EI) m/z calcd for $C_7H_{16}IOSi$ ([M−(*t*-Bu)]$^+$) 271.0015, found 271.0014.

4.1.9. Preparation of ent-**16** and (R)-**17**

These compounds were obtained from *ent*-**15** in the same manners as those described for **16** and (*S*)-**17**. The IR and NMR spectra of *ent*-**16** and (*R*)-**17** were identical with those of **16** and (*S*)-**17**, respectively. Compound *ent*-**16**: $[\alpha]_D^{24}$ +7.67 (*c* 0.920, $CHCl_3$), lit.[23] $[\alpha]_D^{24}$ +8.07 (*c* 1.05, $CHCl_3$); HRMS (FAB) m/z calcd for $C_{11}H_{27}O_2Si$ ([M+H]$^+$) 219.1780, found 219.1789. Compound (*R*)-**17**: $[\alpha]_D^{24}$ +9.32 (*c* 1.11, $CHCl_3$), lit.[23] $[\alpha]_D^{25}$ +10.6 (*c* 1.11, $CHCl_3$); HRMS (EI) m/z calcd for $C_7H_{16}IOSi$ ([M−(*t*-Bu)]$^+$) 271.0015, found 271.0016.

4.1.10. 5-[(S)-4-tert-Butyldimethylsiloxy-3-methylbutoxy]-7-methoxy-4,6-dimethyl1(3H)-isobenzofuranone (**18**)

To a stirred mixture of **10** (235 mg, 1.13 mmol) and K_2CO_3 (562 mg, 4.06 mmol) in acetone (30 ml) was added a solution of (*S*)-**17** (373 mg, 1.14 mmol) in acetone (5 ml) at room temperature, and the mixture was refluxed overnight in the dark. After being cooled, the mixture was filtered, and the filtrate was concentrated in vacuo. The residue was diluted with water and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/EtOAc=10:1) to give 416 mg (90%) of **18** as a colorless oil. $[\alpha]_D^{28}$ −1.4 (*c* 0.900, $CHCl_3$); IR ν 1761 (s), 1602 (m), 1138 (m), 1102 (s), 836 (s); ^{1}H NMR (500 MHz) δ 0.04 (6H, S), 0.89 (9H, s), 0.97 (3H, d, J=6.3 Hz), 1.58–1.65 (1H, m), 1.84–1.93 (1H, m), 1.98–2.05 (1H, m), 2.16 (3H, s), 2.22 (3H, s), 3.47 (1H, dd, J=9.8, 5.9 Hz), 3.51 (1H, dd, J=9.8, 5.9 Hz), 3.82–3.89 (2H, m), 4.02 (3H, s), 5.12 (2H, s); ^{13}C NMR (125 MHz) δ −5.43, −5.41, 9.6, 11.6, 16.8, 18.3, 25.9 (3C), 32.8, 33.8, 62.2, 68.1, 68.3, 71.7, 112.2, 120.0, 125.4, 146.0, 156.6, 162.3, 169.1; HRMS (FAB) m/z calcd for $C_{22}H_{37}O_5Si$ ([M+H]$^+$) 409.2410, found 409.2412.

4.1.11. 5-[(S)-4-Hydroxy-3-methylbutoxy]-7-hydroxy-4,6-dimethyl-1(3H)isobenzofuranone (**19**)

To a stirred suspension of MgI_2 in Et_2O/toluene, prepared from Mg (5.8 mg, 0.24 mmol) and I_2 (30 mg, 0.12 mmol) in Et_2O/toluene (1:2, 3 ml), was added a solution of **18** (81.5 mg, 0.199 mmol) in Et_2O/toluene (1:2, 3 ml) at room temperature, and the mixture was refluxed for 2.5 h in the dark. To the mixture was added a solution of 12 M HCl aq (0.18 ml) in MeOH (1.5 ml) at room temperature, and the resulting mixture was stirred for 1 h before being quenched

with $NaHCO_3$ aq and extracted with EtOAc. The extract was successively washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over SiO_2 (hexane/EtOAc=2:1) to give 49.4 mg (88%) of **19** as a white solid, recrystallization of which from hexane/EtOAc afforded colorless needles: mp 88.5–89.5 °C; $[\alpha]_D^{28}$ −7.34 (*c* 0.730, $CHCl_3$); IR *ν* 3430 (br m), 1731 (s), 1623 (m), 1325 (m), 1152 (m), 1104 (m); ^{1}H NMR (500 MHz) *δ* 1.04 (3H, d, *J*=6.8 Hz), 1.62 (1H, br s, OH), 1.66–1.73 (1H, m), 1.93–2.07 (2H, m), 2.14 (3H, s), 2.20 (3H, s), 3.58 (1H, dd, *J*=10.7, 5.9 Hz), 3.60 (1H, dd, *J*=10.7, 5.9 Hz), 3.88 (2H, t, *J*=6.6 Hz), 5.20 (2H, s), 7.69 (1H, br s, OH); ^{13}C NMR (125 MHz) *δ* 8.7, 11.5, 16.7, 33.0, 33.9, 68.0, 70.0, 71.3, 105.9, 116.5, 118.5, 143.4, 153.6, 162.8, 173.0; HRMS (FAB) *m*/*z* calcd for $C_{15}H_{21}O_5$ ($[M+H]^+$) 281.1389, found 281.1396.

4.1.12. (S)-4-[(1,3-Dihydro-7-hydroxy-4,6-dimethyl-1-oxo-5-isobenzofuranyl)oxy]-2-methylbutanoic acid [(S)-2]

To a stirred solution of **19** (13.9 mg, 49.5 μmol) in CH_2Cl_2 (0.5 ml) were successively added TEMPO (1.5 mg, 9.6 μmol) and $PhI(OAc)_2$ (39.8 mg, 0.124 mmol) at room temperature. After being stirred for 1.5 h, the mixture was poured into water and extracted with CH_2Cl_2. The extract was successively washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was passed through a short column of SiO_2 using hexane/EtOAc (3:1) as the eluant, and the eluted solution was concentrated in vacuo to give an aldehyde (13.5 mg) as a pale yellow oil, which was then mixed with *t*-BuOH (1.8 ml), 20% NaH_2PO_4 aq (0.13 ml), and 2-methyl-2-butene (2 ml). To the mixture was added $NaClO_2$ (30.7 mg, 0.339 mmol) at room temperature, and the resulting mixture was stirred for 1.5 h in the dark. The mixture was poured into water and extracted with EtOAc. The extract was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed over SiO_2 ($CHCl_3$/MeOH=50:1) to give 11.2 mg (77%) of (*S*)-**2** as a white solid, recrystallization of which from H_2O/MeOH afforded colorless prisms: mp 101.0–101.5 °C; $[\alpha]_D^{27}$ +6.8 (*c* 0.45, MeOH), lit.[4] $[\alpha]_D^{24}$ +6.2 (*c* 0.36, MeOH); IR *ν* 3231 (br m), 1739 (vs), 1331 (m), 1153 (m), 1077 (s), 1040 (m); ^{1}H NMR (600 MHz) *δ* 1.32 (3H, d, *J*=7.0 Hz), 1.92–1.98 (1H, m), 2.12 (3H, s), 2.17 (3H, s), 2.22–2.28 (1H, m), 2.83–2.89 (1H, m), 3.85 (2H, t, *J*=6.3 Hz), 5.19 (2H, s); ^{13}C NMR (150 MHz) *δ* 8.7, 11.5, 17.4, 33.8, 36.1, 70.1, 70.5, 106.1, 116.5, 118.6, 143.5, 153.8, 162.6, 173.0, 180.9; HRMS (FAB) *m*/*z* calcd for $C_{15}H_{19}O_6$ ($[M+H]^+$) 295.1182, found 295.1189.

4.1.13. Synthesis of ent-18, ent-19, and (R)-2

These compounds were obtained from (*R*)-**17** in the same manners as those described for **18**, **19**, and (*S*)-**2**. The IR and NMR spectra of *ent*-**18**, *ent*-**19**, and (*R*)-**2** were identical with those of **18**, **19**, and (*S*)-**2**, respectively. Compound *ent*-**18**: $[\alpha]_D^{25}$ +2.0 (*c* 0.940, $CHCl_3$); HRMS (FAB) *m*/*z* calcd for $C_{22}H_{37}O_5Si$ ($[M+H]^+$) 409.2410, found 409.2414. Compound *ent*-**19**: $[\alpha]_D^{25}$ +7.43 (*c* 0.740, $CHCl_3$); HRMS (FAB) *m*/*z* calcd for $C_{15}H_{21}O_5$ ($[M+H]^+$) 281.1389, found 281.1388. Compound (*R*)-**2**: $[\alpha]_D^{24}$ −7.5 (*c* 0.23, MeOH); HRMS (FAB) *m*/*z* calcd for $C_{15}H_{19}O_6$ ($[M+H]^+$) 295.1182, found 295.1188.

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