Regular Article

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Asymmetric Total Syntheses and Structure Elucidations of (+)-Eurotiumide F and (+)-Eurotiumide G

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Asymmetric total syntheses of dihydropyran containing natural products, (+)-eurotiumide F and (+)-eurotiumide G have been described. These total syntheses revealed the absolute configuration of eurotiumide F and G, and confirmed the reported structure of eurotiumide F and revised the reported structure of eurotiumide G. Highlight of these syntheses is thermal rearrangement with 4-methoxyisochroman-1-one derivative having propargyl ether on phenolic ether under thermal condition to construct dihydropyran ring. X-Ray crystallographic analysis of (+)-eurotiumide G clarified the stereochemistry at the C1-position.

Key words total synthesis; natural product; dihydropyran

Introduction

Marine-derived fungi have been recognized as excellent sources of a wide variety classes of secondary metabolites, and a number of useful natural products are isolated and identified by the efforts of a lot of researchers in the field of natural products chemistry.[1–3] It is easy to understand that these natural products have a huge potential to become drugs from the fact that some of marine-derived natural products, such as eribulin[4–6] and trabectedin[7,8] have been used as in clinical sites today. In 2014, Wang and colleagues isolated and identified some dihydroisocoumarin derivatives and related compounds as racemate, named eurotiumides, from gorgonian-derived fungus, *Eurotium* sp. XS-200900E6[9] (Fig. 1). Among them, they reported that eurotiumide A (**1**) displayed potent antimicrobial activities against several bacteria, and eurotiumide B (**2**) exhibited the useful antifouling activity against barnacle *Balanus amphitrite*. Wang and colleagues separated the racemic eurotiumides by chiral HPLC and obtained both enantiomers of eurotiumide A (**1**), B (**2**), C, D, and E, respectively. The absolute configurations of their C3-position were proposed by the comparison of the related known compounds.[10–12] Recently, we reported the first asymmetric total syntheses of eurotiumide A (**1**) and B (**2**), and determined the absolute configuration of both two compounds.[13] Moreover, our total syntheses also revised the relative stereochemistry between C3 and C4 of eurotiumide A (**1**) and eurotiumide B (**2**), respectively. On the other hand, eurotiumide F (**3**) and eurotiumide G (**4**), which have dihydropyran ring and methoxy lactol moiety, were also isolated as racemate from the same fungus. However, chiral HPLC separation of these compounds could not be succeeded and the absolute configurations and optical properties of them are still unknown. Natural products having dihydropyran ring moiety are reported that they displayed unique biological activities.[14–20] Eurotiumide F (**3**) and G (**4**) were evaluated the antimicrobial activities and they displayed medium antimicrobial activities against several strains. As our continuous research, we conducted the asymmetric total syntheses of eurotiumide F (**3**) and eurotiumide G (**4**) for elucidating the asymmetric centers of C3- and C4-positions of them, and evaluate the further investigation of biological activities.

Results

Our synthetic plan is shown in Chart 1. Methoxy lactol moiety, which is the common characteristic structure of eurotiumide F (**3**) and G (**4**), could be constructed by reduction of the corresponding lactones **5** and **6**, and subsequent replacement of the hydroxy group of lactol to methoxy group in acidic MeOH. **5** and **6** are diastereomers at the C3-position, and **5** would be easily obtained from **6** by the sequential hydrolysis and intramolecular Mitsunobu reaction. Dihydropyran moiety of **6** could be constructed by thermal rearrangement with dimethyl propargyl ether compound **7**, which would be obtained from *cis* 4-methoxyisochroman-1-one compound **8** having (3*S*,4*S*) stereochemistry.

In our recent total syntheses of (–)-eurotiumide A (**1**) and (+)-eurotiumide B (**2**),[13] **8** was one of the key compound

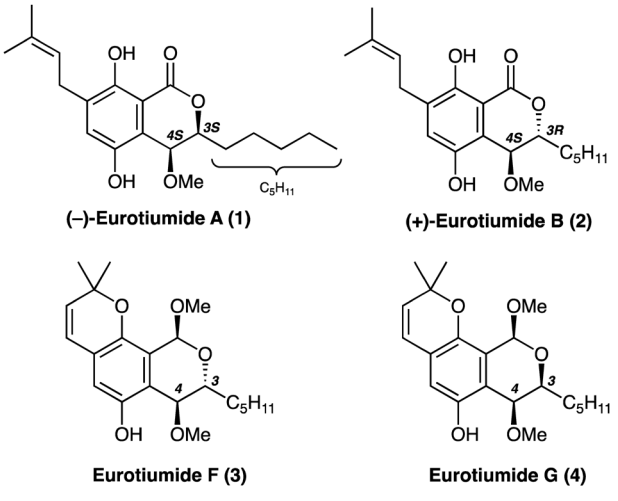

Fig. 1. Reported Structures of Eurotiumide A, B, F, and G

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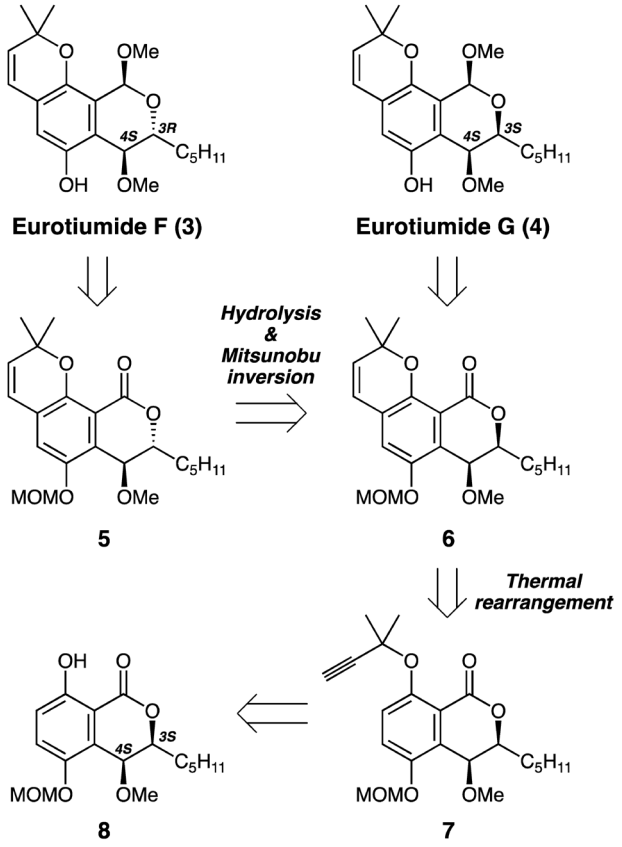


Chart 1. Retrosynthesis of Eurotiumide F and G

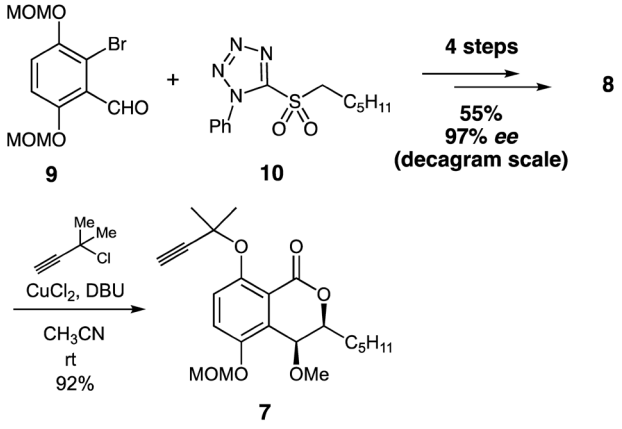
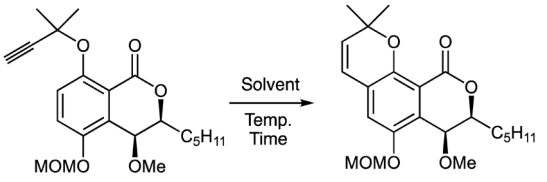


Chart 2. Preparation of Propargyl Derivative **7**

which could be prepared in decagram scale and good enantiomeric purity in 4 steps from aldehyde **9**[21,22] and 1-phenyl-1*H*-tetrazol-5-yl (PT)-sulfone **10**, by Julia–Kocienski olefination, asymmetric Shi epoxidation,[23] BF_3-OEt_2 mediated epoxide opening, and Pd-catalyzed C1 insertion/lactonization cascade reaction[24–27] (Chart 2). The propargyl moiety was introduced by the treatment of **8** with 3-chloro-3-methylbut-1-yne in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and catalytic amount of $CuCl_2$ to afford propargyl derivative **7** in good yield.[28]

With the desired propargyl derivative **7** in hand, we turned our attention to construction of the dihydropyran ring with thermal rearrangement[29–31] (Table 1). First, we heated **7** in benzene under reflux condition, however, the desired dihydropyran derivative **6** was not obtained at all. Next, we changed solvent from benzene to toluene. As a result, **6** was obtained in 65% (entry 2). Moreover, using xylene as a solvent improved the yield (90%) and shorten the reaction time (entry 3). Finally, we found that treatment of **7** in 1,2-dichlorobenzene at 160°C for 3 h gave the desired **6** in quantitative yield (entry 4). We also tried to use microwave condition (200°C, 300W), and observed the dramatically acceleration effect of this thermal rearrangement, and the reaction completed within 10 min in quantitative yield (entry 5).

Table 1. Examination of Thermal Rearrangement



Entry	Solvent	Temp. (°C)	Time (h)	Yield (%)
1	Benzene	Reflux (80)	48	N.R.
2	Toluene	Reflux (110)	72	65
3	Xylene	Reflux (140)	6	90
4	*o*-Dichlorobenzene	Reflux (160)	3	Quant
5	*o*-Dichlorobenzene	200 (MW condition)	0.2	Quant

Next, we constructed the lactol moiety by the reduction of lactone **6** (Chart 3). Treatment of **6** with 1 equiv of diisobutylaluminium hydride (DIBAL) afforded the desired lactol **11** as a sole isomer in good yield. The stereochemistry at the C1-position was assigned as *R* configuration by the careful nuclear Overhauser effect spectroscopy (NOESY) experiment between 1-OH and H-3, and this C1-stereochemistry is considered to be the result of the convergence to the more thermodynamically stable configuration during the treatment of the saturated aqueous Rochelle salt solution (see details in Experimental).[32] Finally, removal of methoxymethyl (MOM) group and displacement of the hydroxy group at the C1-position to a methoxy group were accomplished by 6 M HCl aq in MeOH to afford (+)-eurotiumide G (**4**) in good yield. All spectral data of our synthetic eurotiumide G are identified with those of the reported data, however, the X-ray crystallographic analysis[33] of the synthetic (+)-eurotiumide G revealed the stereochemistry of the C1-position as *R* configuration, not as *S* configuration reported by Wang *et al.* and we could determine the all stereochemistries of (+)-eurotiumide G (**4**) as (1*R*,3*S*,4*S*).

Toward synthesis of eurotiumide F (**3**), first, we transformed *cis* configuration of the C3/C4-positions to *trans* configuration of them. Hydrolysis of **6** and subsequent intramolecular Mitsunobu reaction gave the corresponding *trans* 4-methoxyisochroman-1-one compound **5**. Reduction of lactone moiety with DIBAL afforded lactol **12** in 79% yield. The stereochemistry among C1-, C3-, and C4-positions were determined as shown in Chart 3 by the NOESY experiment and this C1-stereoselectivity would be attributed that DIBAL reduced the lactone moiety of **5** from the opposite side of the 4-OMe group. However, treatment of **12** with 6 M HCl aq in MeOH for displacement of hydroxyl group at the C1-position to a methoxy group and the subsequent deprotection of MOM group did not give eurotiumide F (**3**), but (–)-eurotiumide G (**13**) was obtained.

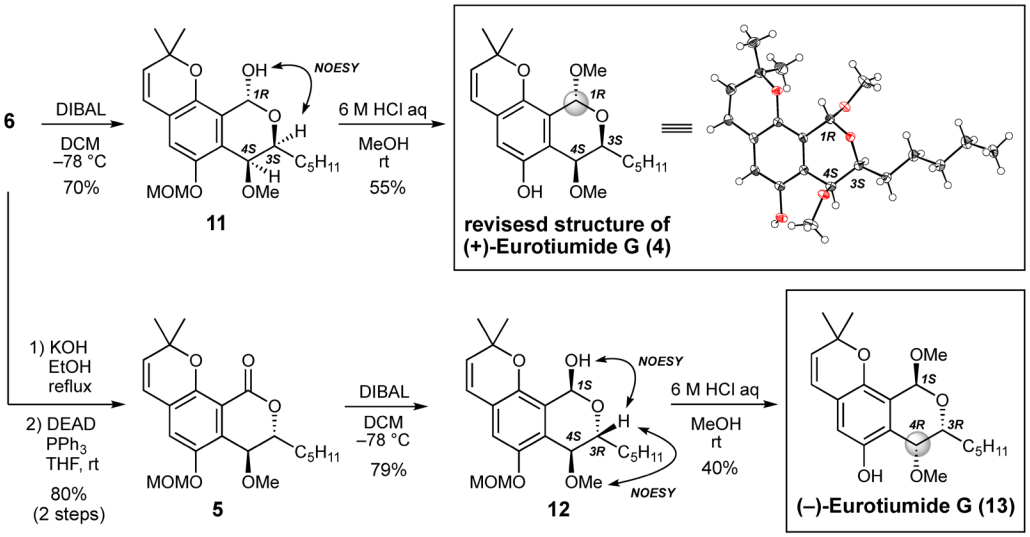


Chart 3. Completion of Total Syntheses of (+)-Eurotiumide G (**4**) and (–)-Eurotiumide G (**13**)

(Color figure can be accessed in the online version.)

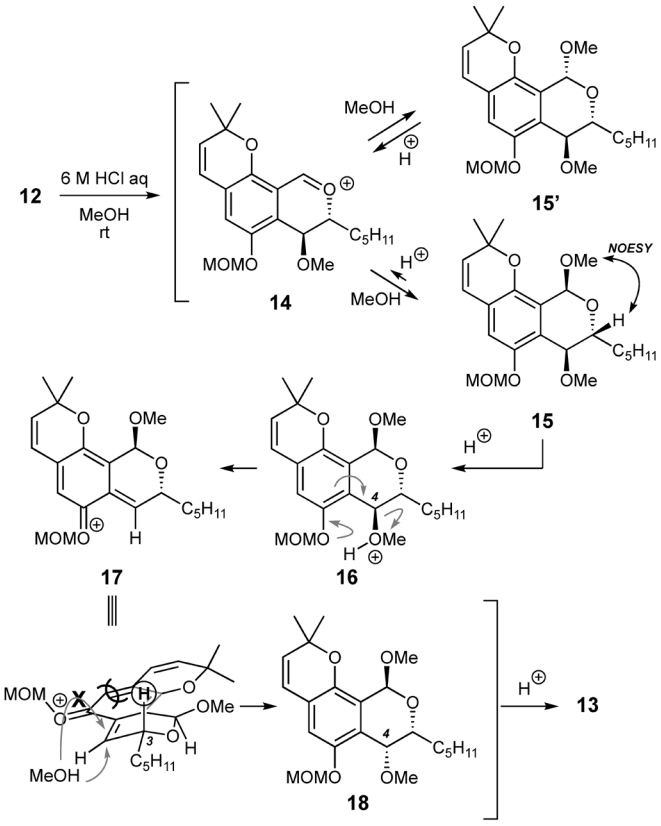


Chart 4. Plausible Mechanism of Generation of (–)-Eurotiumide G (**13**)

The plausible mechanism of generation of **13** is shown in Chart 4. The C1-lactol moiety of **12** is easily transformed to oxonium cation intermediate **14**. Then MeOH attacks at the C1-position and the more stable methoxy lactol **15** having all equatrial substituents was formed as the result of equilibrium with **15′**.[34] The Density Functional Theory (DFT) calculation (Method/Basis set b3lyp/6-31 + g(d,p)) displayed that **15** is more stable than **15′** for 3.79 kcal/mol. Next, an *ortho*-quinone methide **17** is generated by elimination of the C4-methoxy group *via* the protonated compound **16**. In this time, the *ortho*-quinone methide **17** is considered to take the most stable conformation when pentyl group occupies the equatorial position. Then, MeOH attacks at the C4-position by avoiding axial hydrogen at the C3-position to afford C4-epimerized lactol **18**. The DFT calculation (Method/Basis set b3lyp/6-31 + g(d,p)) displayed that **18** is a little more stable than **15** for 0.87 kcal/mol. Finally, the cleavage of MOM group affords (–)-eurotiumide G (**13**). In other words, **13** was obtained as the result of thermodynamically equilibrium at the C1-position and kinetically nucleophilic attack at the C4-position, respectively.

This isomerization at the C4-position seemed to be completed during the deprotection of MOM group. Based on our previous research of total syntheses of **1** and **2**, we found that the isomerization at the C4-position is quite slow under acidic condition with 4-methoxyisochroman-1-one derivative. To avoid this isomerization, we decided to change the order of reduction of lactone moiety and deprotection of MOM group (Chart 5). Treatment of **5** with 6 M HCl aq in MeOH removed MOM group and DIBAL reduction occurred from the opposite side of the 4-OMe group to afford lactol **20**. Finally, displacement of a hydroxy group at the C1-position to a methoxy group was conducted under mild condition, and the treatment of **20** with 0.4 M HCl aq in MeOH at 0°C afforded desired (+)-eurotiumide F (**3**) in moderate yield without isomerization at the C4-position. All spectral data of synthetic eurotiumide F are matched with those of the reported data of eurotiumide F. The absolute configurations of the C1-, C3-, C4-positions of (+)-eurotiumide F were assigned as (1*S*,3*R*,4*S*) by the NOESY experiment.

In our previous total syntheses of eurotiumide A (**1**) and B (**2**), we found that **1** and **2** emitted quite strong fluorescence and the possibility for using natural fluorescent probe. We also evaluated the fluorescent properties of **3** and **4**, respectively. Although they emitted weak violet fluorescence, they were not enough to use as natural fluorescent probes.

In conclusion, we achieved the asymmetric total syntheses of (+)-eurotiumide F and (+)-eurotiumide G involving thermal rearrangement for constructing dihydropyran ring in 7 and 4 steps from the known chiral *cis* 4-methoxyisochroman-1-one compound **8**, respectively. Their absolute stereochemis-

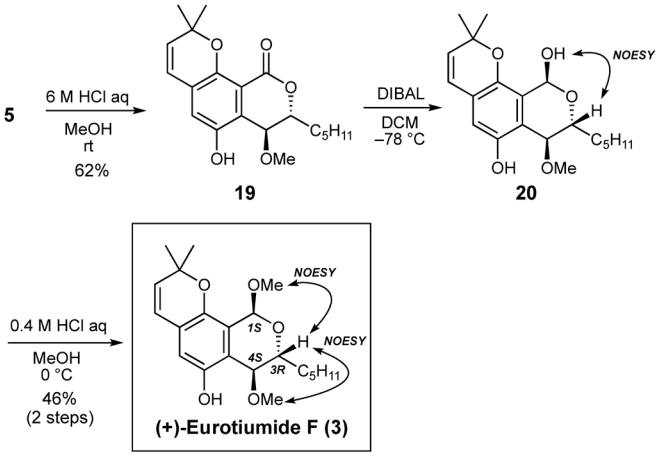


Chart 5. Completion of Total Synthesis of (+)-Eurotiumide F (**3**)

tries were determined by using optically active intermediate **6** and relative stereochemistry of the C1-, C3-, and C4-positions of **3** could be assigned by NOESY experiment. Moreover, the X-ray crystallographic analysis of (+)-**4** revealed the correct stereochemistry at the C1-position and revised the reported structure of **4**. We also found that **3** and **4** emit weak violet fluorescence under UV irradiation. Further investigation of antimicrobial activities is underway in our laboratory.

Experimental

Preparation of Dimethyl Propargyl Ether Compound 7 To a solution of *cis* 4-methoxyisochroman-1-one compound **8** (150.0 mg, 0.462 mmol) in CH_3CN (12.0 mL) were added $CuCl_2$ solution (0.85 mL, 0.00231 mmol, 0.0027 M in CH_3CN), DBU (0.242 mL, 1.62 mmol), and 3-chloro-3-methyl-1-butyne (0.18 mL, 1.62 mmol) at 0°C, successively. After stirring for 12 h at room temperature, the reaction was quenched by adding saturated aqueous NH_4Cl. The mixture was extracted with EtOAc (x3) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc–hexane = 5 : 95 to 10 : 90) to give propargyl derivative **7** (166.1 mg, 92%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.54 (1H, d, J = 9.4 Hz), 7.32 (1H, d, J = 9.4 Hz), 5.22 (2H, s), 4.61 (1H, d, J = 1.6 Hz), 4.22 (1H, ddd, J = 8.5, 5.8, 1.6 Hz), 3.51 (3H, s), 3.31 (3H, s), 2.53 (1H, s), 2.05 (1H, m), 1.80 (1H, m), 1.70 (3H, s), 1.67 (3H, s), 1.46–1.33 (5H, m), 0.90 (3H, t, J = 6.8 Hz). ^{13}C-NMR ($CDCl_3$, 125 Hz) δ: 161.7, 151.2, 149.5, 128.4, 124.7, 120.6, 118.8, 95.2, 86.5, 80.5, 74.7, 74.1, 68.6, 56.7, 56.4, 31.6, 30.6, 29.6, 28.7, 24.9, 22.5, 14.0. IR (KBr) cm^{-1}: 3256, 2373, 1734, 1480, 1258, 1221, 1125. High resolution (HR)-MS (electrospray ionization (ESI)) m/z [M + Na]: 413.1942 (Calcd for $C_{22}H_{30}O_6Na$: 413.1940). $[\alpha]_D^{23}$ +115.0 (c 0.95, CH_2Cl_2, 97% enantiomeric excess (*ee*)).

Preparation of Dihydropyran Derivative 6 with Thermal Condition A solution of alkyne propargyl derivative **7** (60.0 mg, 0.155 mmol) in *o*-dichlorobenzene (15 mL) was stirring at 160°C for 3 h. After the mixture was cooled to room temperature, the reaction mixture was directly purified by silica gel column chromatography (EtOAc–hexane = 10 : 90) to give dihydropyran derivative **6** (60.0 mg, quant) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.03 (1H, s), 6.29 (1H, d, J = 9.8 Hz), 5.76 (1H, d, J = 9.8 Hz), 5.17 (1H, d, J = 7.2 Hz), 5.16 (1H, d, J = 7.2 Hz), 4.57 (1H, d, J = 1.2 Hz), 4.23 (1H, ddd, J = 8.3, 5.8, 1.6 Hz), 3.50 (3H, s), 3.29 (3H, s), 2.03 (1H, m), 1.79 (1H, m), 1.55 (3H, s), 1.45–1.31 (6H, m), 1.42 (3H, s), 0.90 (3H, t, J = 6.8 Hz). ^{13}C-NMR ($CDCl_3$, 100 Hz) δ: 161.4, 149.8, 147.1, 133.2, 127.5, 124.0, 121.6, 117.1, 114.0, 95.5, 80.5, 77.3, 68.5, 56.5, 56.3, 31.6, 30.7, 28.0, 27.8, 25.0, 22.6, 14.0. IR (KBr) cm^{-1}: 1732, 1600, 1460, 1434, 1293, 1153. HR-MS (ESI) m/z [M + H]: 391.2110 (Calcd for $C_{22}H_{31}O_6$: 391.2121). $[\alpha]_D^{23}$ +189.9 (c 0.99, CH_2Cl_2, 97% *ee*).

Preparation of Dihydropyran Derivative 6 with Microwave Condition A solution of alkyne propargyl derivative **7** (4.8 mg, 0.0123 mmol) in *o*-dichlorobenzene (1.2 mL) was stirring at 200°C with microwave (300 W) for 10 min. After the mixture was cooled to room temperature, the reaction mixture was directly purified by silica gel column chromatography (EtOAc–hexane = 10 : 90) to give dihydropyran derivative **6** (4.8 mg, quant) as a yellow oil. These microwave-irradiation reaction was carried out with CEM Discover™SP. All reactions were carried out in sealed heavy-walled Pyrex tubes with constant temperature, pressure, and irradiation power (300 W).

Preparation of Lactol 11 To a solution of dihydropyran derivative **6** (15.4 mg, 0.0394 mmol) in CH_2Cl_2 (0.31 mL) was added DIBAL solution (77 μL, 0.0789 mmol, 1.02 M in Hexane) at −78°C. After stirring for 15 min, saturated aqueous Rochelle salt (0.60 mL) was added to the reaction mixture. After the mixture was stirring for additional 20 min at 0°C, the mixture was extracted with EtOAc (x3) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by preparative thin layer chromatography (EtOAc–hexane = 30 : 70) to give lactol **11** (10.9 mg, 70%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 6.81 (1H, s), 6.28 (1H, d, J = 9.8 Hz), 6.13 (1H, s), 5.65 (1H, d, J = 9.8 Hz), 5.17 (1H, d, J = 6.5 Hz), 5.15 (1H, d, J = 6.5 Hz), 4.29 (1H, d, J = 2.0 Hz), 4.15 (1H, ddd, J = 7.8, 6.3, 1.5 Hz), 3.49 (3H, s), 3.45 (3H, s), 2.83 (1H, s), 1.85 (1H, m), 1.77 (1H, m), 1.47 (1H, m), 1.45 (3H, s), 1.42–1.35 (5H, m), 1.38 (3H, s), 0.91 (3H, t, J = 7.0 Hz). ^{13}C-NMR ($CDCl_3$, 125 Hz) δ: 149.2, 144.2, 131.8, 124.5, 123.5, 122.1, 121.7, 112.1, 95.2, 88.3, 76.5, 71.3, 68.8, 58.1, 56.0, 31.9, 30.7, 28.0, 27.6, 25.5, 22.6, 14.1. IR (KBr) cm^{-1}: 3412, 1731, 1474, 1436, 1272, 1110. HR-MS (ESI) m/z [M + H]: 393.2271 (Calcd for $C_{22}H_{33}O_6$: 393.2277). $[\alpha]_D^{23}$ +15.0 (c 0.34, CH_2Cl_2, 97% *ee*).

Preparation of (+)-Eurotiumide G (4) To a solution of lactol **11** (6.7 mg, 0.0171 mmol) in MeOH (1.3 mL) was added 6 M aqueous HCl (0.43 mL) at 0°C. After stirring for 1.5 h at room temperature, the reaction was quenched by adding saturated aqueous $NaHCO_3$. The mixture was extracted with EtOAc (x3) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by preparative thin layer chromatography (EtOAc–hexane = 30 : 70) to give (+)-eurotiumide G (**4**) (3.4 mg, 55%) as a white solid. mp. 111°C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 6.59 (1H, s), 6.28 (1H, d, J = 9.6 Hz), 6.02 (1H, s), 5.67 (1H, d, J = 9.6 Hz), 5.56 (1H, s), 4.44 (1H, d, J = 3.6 Hz), 4.21 (1H, td, J = 7.0, 3.6 Hz), 3.54 (3H, s), 3.18 (3H, s), 1.80 (2H, q, J = 7.2 Hz), 1.61 (1H, m), 1.51 (1H, m), 1.44–1.34 (10H, m), 0.93 (3H, t, J = 7.0 Hz). ^{13}C-NMR ($CDCl_3$, 125 Hz) δ: 149.0, 143.3, 132.2, 124.0, 122.4, 122.1,

117.7, 113.1, 95.2, 75.9, 70.1, 68.7, 55.7, 53.7, 31.8, 30.2, 27.8, 27.3, 25.8, 22.6, 14.1. IR (KBr) cm^{-1}: 3339, 1639, 1441, 1261, 1110, 1055. HR-MS (ESI) *m/z* [M + H]: 363.2174 (Calcd for $C_{21}H_{31}O_5$: 363.2171). $[\alpha]_D^{23}$ +20.3 (*c* 0.81, CH_2Cl_2, 97% *ee*).

Single crystals of **4**, obtained by slow evaporation of a pentane solution of **4**, were selected and fitted onto a glass fiber and measured at −173°C with a Bruker Apex II ultra diffractometer using MoKα radiation. Data correction and reduction were performed with the crystallographic package Apex3. The structure was solved and refined with the Bruker SHELXTL software package, using the space group $P2_12_12_1$ with Z = 4 for the formula unit $C_{21}H_{30}O_5$. The final anisotropic full-matrix least-squares refinement on F^2 with 241 variables converged at R1 = 3.49%, for the observed data and wR2 = 7.82% for all data. The goodness-of-fit was 0.992. The ORTEP plot was obtained by the program PLATON (A. L. Spek, 2009).

Preparation of *trans* 4-Methoxyisochroman-1-one Compound 5 To a solution of dihydropyran **6** (138.6 mg, 0.355 mmol) in EtOH (12 mL) was added aqueous KOH (4.5 mL, 2.25 mmol, 0.5 M in H_2O) at room temperature. After stirring for 20 min at 90°C, the reaction mixture was cooled to 0°C and neutralized to pH 5.0 with 1 M aqueous HCl. EtOH was removed under reduced pressure and the aqueous layer was extracted with EtOAc (x3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford carboxylic acid as a crude product. To a solution of the crude residue in THF (3.6 mL) was added PPh_3 (279.4 mg, 1.07 mmol) and diethylazodicarboxylate (0.45 mL, 0.994 mmol, 2.2 M in toluene) at 0°C. After stirring for 20 min at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc–hexane = 10 : 90 to 20 : 80) to give *trans* 4-methoxyisochroman-1-one compound **5** (111.5 mg, 80%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.03 (1H, s), 6.29 (1H, d, J = 9.8 Hz), 5.77 (1H, 9.8 Hz), 5.18 (1H, d, J = 6.8 Hz), 5.15 (1H, d, J = 6.8 Hz), 4.71 (1H, ddd, J = 7.8, 6.0, 0.8 Hz), 4.60 (1H, dd, J = 1.2, 0.8 Hz), 3.49 (3H, s), 3.34 (3H, s), 1.50 (1H, m), 1.52 (3H, s), 1.47 (3H, s), 1.39–1.14 (7H, m), 0.85 (3H, t, J = 6.8 Hz). ^{13}C-NMR ($CDCl_3$, 100 Hz) δ: 159.8, 149.7, 148.0, 133.3, 125.5, 124.2, 121.7, 117.7, 113.9, 95.5, 80.2, 70.1, 56.2, 56.0, 32.2, 31.4, 27.85, 27.77, 25.3, 22.4, 13.9. IR (KBr) cm^{-1}: 1730, 1638, 1459, 1434, 1299, 1139. HR-MS (ESI) *m/z* [M + H]: 391.2116 (Calcd for $C_{22}H_{31}O_6$: 391.2121). $[\alpha]_D^{23}$ +175.9 (*c* 1.03, CH_2Cl_2, 97% *ee*).

Preparation of Lactol 12 To a solution of *trans* 4-methoxyisochroman-1-one compound **5** (13.6 mg, 0.0348 mmol) in CH_2Cl_2 (0.27 mL) was added DIBAL solution (68 μL, 0.0697 mmol, 1.02 M in Hexane) at −78°C. After the mixture was stirring for 15 min, saturated aqueous Rochelle salt (0.6 mL) was added to the reaction mixture. After stirring for 20 min at 0°C, the mixture was extracted with EtOAc (x3) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by preparative thin layer chromatography (EtOAc–hexane = 30 : 70) to give lactol **12** (10.8 mg, 79%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.78 (1H, s), 6.28 (1H, d, J = 9.8 Hz), 6.12 (1H, d, J = 11.2 Hz), 5.68 (1H, d, J = 9.8 Hz), 5.16 (1H, d, J = 6.6 Hz), 5.13 (1H, d, J = 6.6 Hz), 4.72 (1H, d, J = 11.2 Hz), 4.61 (1H, d, J = 1.6 Hz), 4.44 (1H, td, J = 6.0, 1.6 Hz), 3.48 (3H, s), 3.32 (3H, s), 1.45 (3H, s), 1.40 (3H, s), 1.34–1.24 (8H, m), 0.85 (3H, t, J = 6.8 Hz). ^{13}C-NMR ($CDCl_3$, 100 Hz) δ: 149.1, 143.2, 132.5, 127.3, 122.4, 122.1, 120.6, 111.9, 95.2, 86.9, 76.4, 75.0, 72.7, 56.0, 55.9, 34.2, 31.7, 27.9, 27.7, 25.0, 22.5, 14.0. IR (KBr) cm^{-1}: 3431, 1638, 1479, 1436, 1265, 1105. HR-MS (ESI) *m/z* [M + H]: 393.2280 (Calcd for $C_{22}H_{33}O_6$: 393.2277). $[\alpha]_D^{23}$ +73.2 (*c* 0.55, CH_2Cl_2, 97% *ee*).

Preparation of (–)-Eurotiumide G (13) To a solution of lactol **12** (5.5 mg, 0.0139 mmol) in MeOH (1.0 mL) was added 6 M aqueous HCl (0.35 mL) at 0°C. After stirring for 4 h at room temperature, the reaction was quenched by adding saturated aqueous $NaHCO_3$. The mixture was extracted with EtOAc (x3) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by preparative thin layer chromatography (EtOAc–hexane = 30 : 70) to give (–)-eurotiumide G (**13**) (2.0 mg, 40%) as a yellow oil. All spectral data except for optical rotation were identified with those of the data of (+)-eurotiumide G (**4**) prepared by us. $[\alpha]_D^{23}$ –22.8 (*c* 0.20, CH_2Cl_2).

Preparation of Free Phenol 19 To a solution of *trans* 4-methoxyisochroman-1-one compound **5** (204.5 mg, 0.524 mmol) in MeOH (39 mL) was added 6 M aqueous HCl (13.1 mL) at 0°C. After stirring for 4 h at room temperature, the reaction was quenched by adding saturated aqueous $NaHCO_3$. The mixture was extracted with EtOAc (x3) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc–hexane = 15 : 85 to 25 : 75) to give free phenol **19** (111.8 mg, 62%) as a yellow amorphous. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.84 (1H, br-s), 6.81 (1H, d, J = 1.6 Hz), 6.25 (1H, d, J = 10.0 Hz), 5.75 (1H, d, J = 10.0 Hz), 4.66 (1H, d, J = 4.0 Hz), 4.63 (1H, m), 3.43 (3H, s), 1.58–1.20 (8H, m), 1.48 (3H, s), 1.46 (3H, s), 0.86 (3H, t, J = 7.0 Hz). ^{13}C-NMR ($CDCl_3$, 100 Hz) δ: 160.7, 149.1, 147.1, 133.5, 124.3, 122.1, 121.4, 119.2, 112.8, 79.8, 77.2, 73.1, 56.3, 32.0, 31.5, 27.7 (overlapped), 25.0, 22.5, 13.9. IR (KBr) cm^{-1}: 3168, 1684, 1598, 1455, 1441, 1305, 1155. HR-MS (ESI) *m/z* [M + H]: 347.1853 (Calcd for $C_{20}H_{27}O_5$: 347.1858). $[\alpha]_D^{23}$ +107.8 (*c* 1.01, CH_2Cl_2, 97% *ee*).

Preparation of Lactol 20 To a solution of free phenolic alcohol **19** (46.0 mg, 0.133 mmol) in CH_2Cl_2 (2.7 mL) was added DIBAL solution (2.0 mL, 1.99 mmol, 1.02 M in Hexane) at −78°C over 2 h with a syringe pump. After stirring for 30 min at the same temperature, saturated aqueous Rochelle salt (6.0 mL) was added to the reaction mixture. After stirring for 20 min at 0°C, the mixture was extracted with EtOAc (x3) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude product. Because lactol **20** was unstable on silica gel, the crude product of **20** was used for the next reaction immediately without further purification. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.38 (1H, s), 6.52 (1H, s), 6.26 (1H, d, J = 9.8 Hz), 6.01 (1H, d, J = 3.8 Hz), 5.65 (1H, d, J = 9.8 Hz), 4.71 (1H, d, J = 9.0 Hz), 4.46 (1H, td, J = 9.0, 2.8 Hz), 3.28 (3H, s), 2.94 (1H, d, J = 3.8 Hz), 1.87 (1H, m), 1.65–1.33 (7H, m), 1.46 (3H, s), 1.36 (3H, s), 0.92 (3H, t, J = 7.2 Hz). ^{13}C-NMR ($CDCl_3$, 100 Hz) δ: 149.4, 142.5, 132.1, 125.1, 122.3, 121.9, 117.7, 113.3, 88.1, 76.3, 75.7, 65.5, 51.7, 31.8 (overlapped), 28.0, 27.4, 24.7, 22.6, 14.1. IR (KBr) cm^{-1}: 3431, 1638, 1459, 1361, 1168, 1130. HR-MS (ESI) *m/z* [M + H]: 349.2018 (Calcd for $C_{20}H_{29}O_5$: 349.2015). $[\alpha]_D^{23}$ +83.6 (*c* 0.64, CH_2Cl_2, 97% *ee*).

Preparation of (+)-Eurotiumide F (3) To a solution of crude product of **20** in MeOH (7.5 mL) was added 0.4 M aqueous HCl (2.5 mL) at 0°C. After stirring for 15 min at the same temperature, the reaction was quenched by adding saturated aqueous $NaHCO_3$. The mixture was extracted with EtOAc (x3) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by preparative thin layer chromatography (EtOAc–hexane = 30 : 70) to give (+)-eurotiumide F (**3**) (22.2 mg, 46% in 2 steps) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.42 (1H, s), 6.51 (1H, s), 6.25 (1H, d, J = 10.0 Hz), 5.64 (1H, d, J = 10.0 Hz), 5.44 (1H, s), 4.69 (1H, d, J = 9.8 Hz), 4.31 (1H, td, J = 9.8, 2.4 Hz), 3.56 (3H, s), 3.24 (3H, s), 1.89 (1H, m), 1.65 (1H, m), 1.55 (1H, m), 1.44 (3H, s), 1.41–1.31 (5H, m), 1.34 (3H, s), 0.93 (3H, t, J = 7.2 Hz). ^{13}C-NMR ($CDCl_3$, 125 Hz) δ: 149.3, 142.9, 132.0, 124.1, 122.4, 122.0, 117.7, 113.2, 95.1, 75.91, 75.89, 64.7, 55.9, 51.2, 31.9, 31.8, 27.8, 27.4, 25.1, 22.7, 14.1. IR (KBr) cm^{-1}: 3395, 1727, 1560, 1460, 1359, 1282, 1132. HR-MS (ESI) m/z [M + H]: 363.2179 (Calcd for $C_{21}H_{31}O_5$: 363.2171). $[\alpha]_D^{23}$ +95.3 (c = 0.96, CH_2Cl_2, 97% *ee*).

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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32) The DFT calculation (b3lyp/6-31 + G(d,p)) supports that **11** would be more stable than that of the C1-enantiomer of **11** for 4.12 kcal/mol.
33) Crystallographic data of **4** have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 1918279. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ U.K. (Fax: +44(0)–1223–336033 or e-mail: deposit@ccdc.cam.ac.uk).
34) During this isomerization, only the methoxy lactol **15** could be detected and determined the stereochemistry at the C1-position by observing the NOESY correlation between 1-OMe and H-3.