Enantioselective Synthesis of Homoisoflavanones by Asymmetric Transfer Hydrogenation and Their Biological Evaluation for Antiangiogenic Activity

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Abstract

Neovascular eye diseases are a major cause of blindness. Excessive angiogenesis is a feature of several conditions, including wet age-related macular degeneration, proliferative diabetic retinopathy, and retinopathy of prematurity. Development of novel anti-angiogenic small molecules for the treatment of neovascular eye disease is essential to provide new therapeutic leads for these diseases. We have previously reported the therapeutic potential of anti-angiogenic homoisoflavanone derivatives with efficacy in retinal and choroidal neovascularization models, although these are racemic compounds due to the C3-stereogenic center in the molecules. This work presents asymmetric synthesis and structural determination of anti-angiogenic homoisoflavanones and pharmacological characterization of the stereoisomers. We describe an

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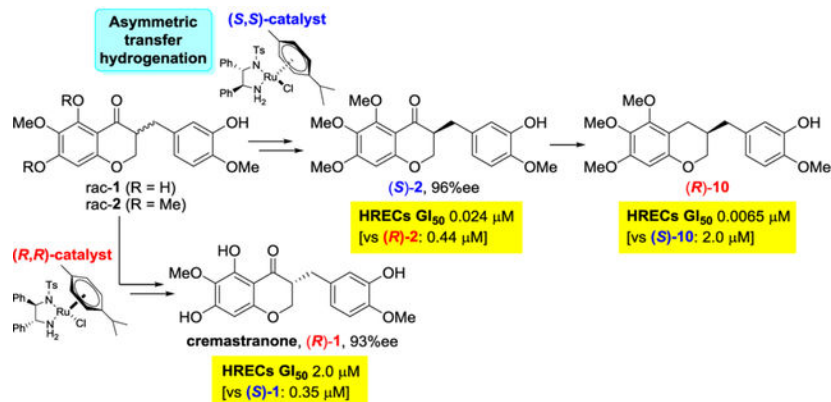[¶]These authors contributed equally.

enantioselective synthesis of homoisoflavanones by virtue of ruthenium-catalyzed asymmetric transfer hydrogenation accompanying dynamic kinetic resolution, providing a basis for the further development of these compounds into novel experimental therapeutics for neovascular eye diseases.

Graphical Abstract

Keywords

Homoisoflavanone; 3-Benzyl-chroman-4-one; 3-Benzyl-chroman-4-ol; Asymmetric transfer hydrogenation; Dynamic kinetic resolution; Angiogenesis; Ocular neovascularization

INTRODUCTION

Angiogenesis, a physiological process in which new blood vessels are formed, is involved in the progression of several diseases such as cancer and neovascular eye disease.[1] These latter diseases, including neovascular ("wet") age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR), and retinopathy of prematurity (ROP), are characterized by abnormal angiogenesis in the posterior segment of the eye (retina and choroid), leading to vascular ischemia and leakage that can result in hemorrhage, edema, and retinal detachment. Retinal and choroidal neovascularization (CNV) are major causes of vision loss in eye disease and have an immense effect on quality of life.[2] Anti-angiogenic therapies targeting the important, proangiogenic vascular endothelial growth factor (VEGF) pathway have been successful in stabilizing, or even improving central vision in many patients.[3] The present anti-VEGF therapies such as ranibizumab, bevacizumab, pegaptanib, and aflibercept are used as standard treatments for neovascular AMD. Although these biologics are now used widely, they have limitations: most target the VEGF pathway only at the level of the VEGF ligand-receptor interaction, approximately 35% of patients with wet AMD are refractive to these biologics[4] and they can also be associated with significant side effects.[5] There are as yet no approved small molecules for treating retinal or choroidal neovascularization, although some promising small molecules are being developed.[6] Thus, novel antiangiogenic small molecules still need to be developed to complement and perhaps combine with existing therapies for wet AMD, PDR and ROP.

Homoisoflavanones consist of a 3-benzylchroman-4-one skeleton and are a small group of natural chromane heterocycles, purified from various plants. Some review articles deal with the plant sources and biological activities of the homoisoflavanones.[7] These natural products show a wide range of biological activities such as anti-inflammatory, antioxidant, anti-microbial, and anticancer activities. There is an antiangiogenic homoisoflavanone, 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)-6-methoxychroman-4-one, called cremastranone (**1**), that has been isolated from the plants *Muscari armeniacum*,[8] *Chionodoxa luciliae*,[9] *Scilla natalensis*,[10] *Merwilla plumbea*,[11] and *Cremastra appendiculata* (D. Don).[12] Cremastranone was reported to inhibit neovascularization through anti-proliferative activity against human umbilical vein endothelial cells (HUVECs). It had a 50% growth inhibitory concentration (GI_{50}) value of 1.5 μM without cytotoxic effect (Figure 1) and reduced retinal neovascularization in the laser-induced choroidal neovascularization (L-CNV) and oxygen induced retinopathy (OIR) mouse models.[13] We successfully synthesized cremastranone (**1**) as a racemic mixture and evaluated its anti-proliferative activity against HUVECs and also the more tissue-relevant human retinal microvascular endothelial cells (HRECs) with GI_{50} values of 0.38 and 0.22 μM, respectively, with slightly more potency than the naturally occurring cremastranone.[14]

So far, our synthetic efforts toward cremastranone (**1**) have positively impacted our phenotype-based drug discovery and target identification program. We sought structural modification to increase antiangiogenic activity.[15] As synthetic analogs of cremastranone were designed and synthesized by A and B ring modification, 3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**2**) and a phenylalanyl-incorporated analog (**3**) were identified that inhibit ocular neovascularization with selectivity for HRECs (GI_{50} of 2.0 and 0.055 μM, respectively), over other cell types as well as promising efficacy in OIR and L-CNV in vivo models.[15,16] We also used these compounds as starting points to identify target(s) by chemical proteomics.[17,18] Our homoisoflavanone-based photoaffinity probes were exploited for binding protein and target validation for antiangiogenic mechanism, leading to target proteins for two different compounds, ferrochelatase (FECH) and soluble epoxide hydrolase (sEH).[19,20]

However, the stereochemistry at the C3 position in cremastranone has not been reported, although the (*R*)-form is known as the configuration of general naturally occurring homoisoflavanones.[7] Moreover, the relationship between biological activity and absolute stereochemistry has not yet been confirmed with enantioenriched compounds obtained by chiral separation or asymmetric synthesis. In addition, 5,6,7-trimethoxyhomoisoflavanones **2** and **3** were synthesized and evaluated as mixtures of two stereoisomers.[17] In the case of the analog **3**, the (*S*,*S*)- and (*R*,*S*)-isomers were named based on two stereogenic centers at C3 and the α-carbon of the *L*-phenylalanyl residue (Figure 1). As each isomer potentially exhibited different pharmacological activity, it was necessary to develop an investigational drug for the treatment of wet AMD from the most potent stereoisomer.

There have been many efforts towards the synthesis of homoisoflavonoids including asymmetric synthesis.[21] To obtain an enantiopure antipode of homoisoflavanone efficiently, the previous synthetic approaches developed by others were studied as follows (Figure 2): 1) Intramolecular Friedel-Crafts acylation of chiral 2-benzyl-3-aryloxypropanoic acid was

adapted for the formation of the 3-benzyl-4-chromanone. Such a chiral carboxylic acid was obtained by lipase-catalyzed reaction of 2-benzyl-1,3-propanediol, followed by Mitsunobu reaction of phenol (Kometani et al.[22]) and by iridium-catalyzed asymmetric hydrogenation of α-aryloxymethylcinnamic acid (Zhou et al.[23]), 2) the introduction of a *p*-methoxybenzyl group (for B ring) by asymmetric aldol reaction with Evans chiral oxazolidinone, followed by the intramolecular etherification to provide chiral 3-benzyl-chromane, which was transformed into the 3-benzyl-4-chromanone by benzylic oxidation (Liu et al.[24]), 3) enantioselective protonation of enol ether derived from racemic homoisoflavanones by phase transfer catalysts (Levacher et al.[25]).

Nevertheless, there are still limitations on asymmetric synthesis of homoisoflavanones with various substituents on the A and B rings. Since we undertook a medicinal chemistry program on antiangiogenic cremastranone, we have been interested in the asymmetric synthesis of cremastranone and the biological effects of chiral homoisoflavanones. Moreover, our synthetic route would be very useful for introducing chemical diversity and for the structural optimization of homoisoflavanone derivatives. In screening the best conditions for asymmetric synthesis of homoisoflavanones, we found that the Metz group,[26] Merck group,[27] and others[28] developed asymmetric syntheses of chroman-4-ones, including flavanone and isoflavanone, by asymmetric transfer hydrogenation using Noyori's catalysts. [29,30] Thus, we envisioned asymmetric transfer hydrogenation of the 3-benzyl-chroman-4-ones **1** and **2** by two Noyori's ruthenium catalysts (RuCl(*p*-cymene)[(*R,R*)-Ts-DPEN], (*R,R*)-**4**, and RuCl(*p*-cymene)[(*S,S*)-Ts-DPEN], (*S,S*)-**4**) and subsequent re-oxidation of the resulting 3-benzyl-chroman-4-ol without racemization to provide each (*R*)- or (*S*)-homoisoflavanone (Scheme 1). It was necessary to optimize the reaction conditions through which the reduction of the enolizable ketone of homoisoflavanone would proceed completely by asymmetric transfer hydrogenation to afford enantiomerically pure 3-benzyl-chroman-4-ol. It would be notable if dynamic kinetic resolution accompanied the asymmetric transfer hydrogenation for the enantioselective synthesis of homoisoflavanones, as shown in a previous work.[26,27,31]

Herein, we describe enantioselective synthesis and structural elucidation of the naturally occurring homoisoflavanone cremastranone with (*R*)-configuration and its (*S*)-enantiomer in which stereochemistry dictates in vitro efficacy against angiogenesis. It was shown that the stereoisomers with an (*S*)-configuration on the C3-position are generally superior to the opposite isomers (natural forms) in terms of antiangiogenic efficacy. In addition, we present an efficient method to access several enantioenriched derivatives of homoisoflavanones and reveal the dynamic kinetic resolution mechanism of asymmetric transfer hydrogenation by time-dependent concentration.

RESULTS AND DISCUSSION

Chemistry

Our initial work was to prepare the ketone substrates for asymmetric transfer hydrogenation (Scheme 2). With two 2-hydroxyacetophenones in hand, 5,6,7-trisubstituted-homoisoflavanones **5a** and **5b** were prepared via our previous method in which *N,N*-dimethylformamide dimethyl acetal was used, followed by catalytic hydrogenation or

LiAlH$_4$ reduction of 4*H*-chromen-4-one.[17,32] The resulting chroman-4-ones **5a** and **5b** were treated with isovanillin and *p*-TsOH, then subsequent catalytic hydrogenation afforded the homoisoflavanones **2** and **6**. To screen the substrates for asymmetric transfer hydrogenation, two benzyl-protected analogs of homoisoflavanones (**7** and **8**) were generated using BnBr and K$_2$CO$_3$ from **2** and **6**, respectively. With four homoisoflavanones in hand, we attempted the synthesis of homoisoflavon-4-ol derivatives by diastereoselective (*cis* or *trans*) and enantioselective (*R*- or *S*-configuration) methods.

First, C4-carbonyl reduction of homoisoflavonoids was carried out using some metal hydride agents to study the chemical yield and the ratio of the *cis*- to *trans*-isomer of 3-benzyl-chroman-4-ol (Table 1).[33] Homoisoflavanones **2** and **7** were treated with NaBH$_4$ or NaBH$_4$/CeCl$_3$ to provide the resulting rac-**9a** and rac-**9c** with *cis/trans* ratio of 3.3:1 and 3.5:1, respectively. The *cis/trans* diastereoselectivity was not improved in the case of general procedures using LiAlH$_4$, DIBAL and Super-H®. Unlike the other metal hydride reagents, L-selectride afforded *cis*-**9a** and *cis*-**9c**, respectively, as a major product selectively with 89% and 99% yield. The structural elucidation of the *cis*- and *trans*-isomers of 3-benzyl-chroman-4-ol rac-**9a** was confirmed by 2D NOESY NMR spectroscopy (Supplementary Figure S1).[34]

For the enantioselective synthesis of 3-benzyl-chroman-4-ol, the asymmetric transfer hydrogenation was performed with a Noyori's ruthenium catalyst, RuCl(*p*-cymene)[(*R*,*R*)-Ts-DPEN], (*R*,*R*)-**4** in order to obtain (3*R*,4*R*)-**9a** selectively (Table 2). Initially, the use of 5 mol% of (*R*,*R*)-**4** or the mixture of KOH and *i*-PrOH as a hydrogen source was ineffective. A mixture of triethylamine and formic acid was chosen as a hydrogen source and ethyl acetate was used as a solvent. Low yields were observed without degassing (entry 1 vs 2). Although the yield was not sufficient under these conditions, the ratio of *cis/trans* and enantioselectivity for *cis*-**9a** was promising (entry 2). The *cis/trans* ratio was calculated with ^{1}H-NMR spectroscopy and enantioselectivity was confirmed by chiral HPLC analysis on Chiralpak AD-3 after the optimization of HPLC conditions with racemic *cis*-**9a**. The 3:1 ratio (v/v) of base and formic acid improved yields based on recovered starting material (entries 4 and 5). However, the chemical yield was not improved with 10–20 mol% of (*R*,*R*)-**4**, regardless of screening solvent, base, and the ratio of base and formic acid. The low reactivity under general asymmetric transfer hydrogenation conditions is due to the steric hindrance of the C5-methoxy on the A ring. Given the above results, we postulated that the yield is limited under various conditions, as (*R*)-homoisoflavanone could react much faster than the (*S*)-form just by kinetic resolution. Fortunately, the reactions with 30 mol% (*R*,*R*)-**4** and (*S*,*S*)-**4** gave rise to excellent yield and enantioselectivity (91–96%, 97%ee). As the carbonyl group in **2** has quite low reactivity due to C5 methoxy group, the chemical yield could increase by loading 30 mol% catalyst and extending the reaction time. Thus, the asymmetric transfer hydrogenation worked with accompanying dynamic kinetic resolution (entries 9–11). The optimized condition was a 3:1 ratio of DBU and formic acid in acetonitrile (entry 10). Similarly, (3*S*,4*S*)-**9a** could be obtained by 30 mol% of (*S*,*S*)-**4** successfully (entry 11). A high proportion of DBU affected the ratio of the *cis/trans* isomers and the chemical yield (entry 12). To our knowledge, there are no reports on the asymmetric transfer hydrogenation of chroman-4-ones that contain a C5-substituent on A ring, but we

succeeded in the asymmetric transfer hydrogenation of a 5-substituted 3-benzyl-chroman-4-one such as 5,6,7-trimethoxy-homoisoflavanone and the NMR spectra of (3*R*,4*R*)-**9a** and (3*S*,4*S*)-**9a** are identical to those of the racemic *cis*-**9a**.

As a means to elucidate the absolute configuration of 3-benzyl-chroman-4-ol's C4 secondary alcohol, the ECD spectra of two enantiopure *cis*-**9a** isomers synthesized by asymmetric transfer hydrogenation using (*R*,*R*)-**4** and (*S*,*S*)-**4** were measured. Following many reports on asymmetric transfer hydrogenation, the two enantiomers of *cis*-**9a** were regarded to be (3*R*,4*R*)-**9a** and (3*S*,4*S*)-**9a**, respectively.[26] The enantiomers (3*R*,4*R*)-**9a** and (3*S*,4*S*)-**9a**, with 98%ee and 93%ee, as determined by chiral HPLC, showed positive and negative optical rotation, respectively. The experimental ECD confirmed that the two compounds have the opposite configuration as enantiomers (Figure 3; Supplementary Table S1). The theoretical calculations of the ECD spectra were performed for a pair of enantiomers of *cis*-**9a** using time-dependent density functional theory (TD-DFT) at the B3LYP functional and the 6–31+G(d) basis set via Gaussian 09. As expected, the calculated ECD spectra of (3*R*,4*R*)-**9a** and (3*S*,4*S*)-**9a** were consistent with their experimental curves. Therefore, the full stereochemistry of two chiral 3-benzyl-chroman-4-ols was obtained by asymmetric transfer hydrogenation was assigned through the ECD and NMR spectra.

With (3*R*,4*R*)-**9a** in hand, we studied the oxidation of the C4 secondary alcohol without racemization to obtain the chiral homoisoflavanone (*R*)-**2** under various conditions such as TPAP/NMO, PCC, Dess-Martin periodinane and Swern oxidation. Unexpectedly, most oxidation reactions did not proceed due to the phenol group on the C3′ position, as was also predicted to affect the synthesis of cremastranone. After introducing a benzyl group to the phenol group at the C3′ and C7 positions, asymmetric transfer hydrogenation of the benzyl-protected ketones (**7** and **8**) as well as the ketone **6** containing two phenol groups was carried out (Table 3). Not surprisingly, the ketone **6** was not converted into the alcohol **9b** under two different conditions using Et₃N and DBU (entries 1 and 2). The chemical yield was low after the treatment of the ketone **7** with 10 mol% of (*R*,*R*)-**4** but the enantioselectivity of the *cis*-isomer was >90%ee (entries 3 and 4). DBU was superior to Et₃N as a base in terms of chemical yield and enantioselectivity, and acetonitrile was used as the preferred solvent rather than ethyl acetate. The carbonyl reduction of **7** with 30 mol% of (*R*,*R*)-**4** and (*S*,*S*)-**4**, DBU and formic acid afforded (3*R*,4*R*)-**9c** and (3*S*,4*S*)-**9c** in 92%–93% yield and 93%ee–97%ee, respectively. The ketone **8**, a precursor of cremastranone, was treated with 30 mol% of (*R*,*R*)-**4** and (*S*,*S*)-**4** to provide (3*R*,4*R*)-**9d** and (3*S*,4*S*)-**9d** in 87%–92% yield and 96%ee–98%ee, respectively.

With the pair of enantiomers of **9c**, the oxidation of secondary alcohol under TPAP and NMO conditions, followed by benzyl deprotection provided the desired chiral (*R*)-**2** and (*S*)-**2** in moderate yield (Scheme 3). Fortunately, the two-step reaction did not cause epimerization. Moreover, chiral (*R*)-**2** and (*S*)-**2** were coupled with two different enantiomers of Boc-phenylalanine under EDCI-mediated conditions to provide four stereoisomers of antiangiogenic homoisoflavanone **3**: (*R*,*R*)-, (*S*,*R*)-, (*R*,*S*)-, and (*S*,*S*)-**3**, respectively. Chiral 3-benzyl-chromanes were considered as attractive derivatives of homoisoflavonoids; a racemic 3-benzyl-chromane **10** reported recently showed potent

activity.[35] Thus, (S)-3-benzyl-chromane (S)-**10** was obtained from (3R,4R)-**9a** by treatment with triethylsilane. Similarly, the enantiomer (R)-**10** was generated from (3S,4S)-**9a**. Triethylsilane was more useful to obtain the enantiopure 3-benzyl-chromane compounds than H_2 and Pd/C for minimizing racemization. Unfortunately, the catalytic hydrogenation of chiral (R)-**2** and (S)-**2** afforded (S)-**10** and (R)-**10** in only 71%ee and 78%ee, respectively.

With (3R,4R)-**9d** and (3S,4S)-**9d** in hand, we explored the asymmetric synthesis of the natural product homoisoflavanone, cremastranone (**1**) for the first time (Scheme 4). Similar to the oxidation issue of (3R,4R)-**9a**, we observed that the alcohol oxidation of (3R,4R)-**9b**, did not work, although various oxidizing reagents were screened. Thus, Ley's oxidation of benzyl-protected (3R,4R)-**9d** was used, followed by BCl_3-mediated deprotection to give the desired cremastranone (**1**) in a quantitative yield. The synthetic cremastranone obtained stereoselectively exhibited a negative rotation with $[\alpha]_D^{20}$ −33.0 (c 0.20, MeOH) and its NMR spectra were identical to those of naturally occurring cremastranone of which the optical rotation was reported with $[\alpha]_D^{20}$ −16.0 (c 0.20, MeOH). These experimental data suggested that naturally occurring cremastranone possesses an (R)-configuration at the C3 position of the 3-benzyl-chroman-4-one skeleton. Starting from (3S,4S)-**9d**, the (S)-enantiomer of cremastranone ((S)-**1**) were obtained in a similar manner.

We also studied the time-concentration of ketone **2** and alcohol **9a** in order to prove the mechanism of asymmetric transfer hydrogenation under our developed conditions. To investigate the dynamic kinetic resolution of rac-**2**, we monitored the progress of (3S,4S)-**9a** formation via asymmetric transfer hydrogenation of (R)-**2** and (S)-**2** with (S,S)-**4** by chiral HPLC (Figure 4). After 6 h, alcohol (3S,4S)-**9a** and (S)-**2** were generated at 34% and 18%, respectively. We speculated that the racemization on the C3-position occurred to generate (S)-**2**, which could be converted to (3S,4S)-**9a** whereas in this reaction using (S,S)-**4**, starting material, (R)-**2** was not directly transformed into the *trans*-isomer (3S,4R)-**9a**. The yield of (3S,4S)-**9a** was increased by 24 h and the enantioselectivity of *cis*-isomer was improved up to 98%ee. After 24 h, the ratio of *cis*- to *trans*-isomer was 10 : 1 and (3R,4R)-**9a** was no more than 2% ((a), Figure 4). The reaction of starting material (S)-**2** using catalyst (S,S)-**4** afforded (3S,4S)-**9a** in 74% yield and 99.9%ee, even after 6 h. As 8%–11% of (R)-**2** was found at 6 and 12 h, it was confirmed that racemization occurred partially. Finally, (3S, 4S)-**9a** was provided in 89% yield and 99.9%ee at 24 h.

Antiangiogenic activity of enantiopure homoisoflavanones

As the absolute configuration of natural cremastranone was assigned as the (R)-form and each pair of enantiomers which are 5,6,7-trisubstituted homoisoflavanones were obtained, we considered the antiangiogenic activity of enantiopure homoisoflavanones as (R)- and (S)-isomers compared with that of racemic mixtures. Previously, the GI_{50} value of cremastranone isolated from *C. appendiculata* against HUVECs was reported as 1.5 μM (Figure 1).[12] In contrast, the growth suppressive activity of racemic cremastranone **1** (synthesized by our group) against HUVECs was GI_{50} = 377 nM in an alamarBlue fluorescence cell proliferation assay (Figure 1).[14a] In addition, synthetic **1** showed a dose-dependent, anti-proliferative activity of GI_{50} = 217 nM against HRECs, a more tissue-relevant, microvascular endothelial cell type. As suggested previously, the somewhat more

pronounced inhibitory effect of synthetic compound **1** over the natural product might be due to differences in the biological assays used or purity of the compound. Between the racemic and enantiopure cremastranones, (*R*)-**1** exhibited somewhat weaker inhibitory activity on cell proliferation for HUVECs and HRECs than that of the racemate and (*S*)-**1**.

The (*S*)-isomer of 5,6,7-trimethoxyhomoisoflavanone derivative **2** had 20-fold higher activity than the (*R*)-isomer, similar to that of cremastranone (Table 4). Among the four stereoisomers of SH-11037 (**3**), the isomer (*S*,*S*)-**3** which was incorporated with the coupling of (*S*)-**2** and Boc-L-Phe-OH showed the strongest inhibition of HREC growth. In antiangiogenic activity of 3-benzyl-chroman-4-ol **9**, the *cis*-disubstituted chromane has activity as strong as **3** but the *trans*-form lost the inhibitory activity in HRECs. In addition, the pair of 3-benzyl-chromanes **10** were evaluated for anti-angiogenic activity. In alamarBlue proliferation assays, racemic and (*R*)-**10** had the most pronounced potency and endothelial-cell selectivity among the antiangiogenic homoisoflavonoids that were made.

Previously, we evaluated the cell specificity of synthetic homoisoflavanones by assessing their anti-proliferative effects on non-endothelial ocular cell lines, 92–1 (uveal melanoma cell line) and Y79 (retinoblastoma cell line), as surrogates for non-target ocular tissues. The anti-proliferative activity of racemic mixture **3** on these non-endothelial cell types was considerably less than that on endothelial cells (GI_{50} = 12 μM and >100 μM for Y79 and 92–1, respectively), suggesting that racemic mixture **3** has pronounced selectivity of antiproliferative effect toward endothelial cells. This trend was also seen for the (*R*)- or (*S*)-isomers, although in general in the current work, Y79 cells were more sensitive than in previous reports, likely reflective of a cell batch effect.

Validation of a potent cremastranone derivative in cell-based assays

After demonstrating that enantiopure homoisoflavanones had anti-proliferative activity against HRECs, we explored HREC migration and tube formation in the presence of anti-angiogenic homoisoflavanones and a comparison between more highly anti-proliferative (*S*)-homoisoflavanones and (*R*)-antipodes. These assays document key properties of endothelial cells and together provide a good model for the angiogenesis that occurs in vivo. During angiogenesis, cells migrate out from pre-existing capillaries to the location of neovessel formation. We assessed the migration ability of HRECs via the scratch-wound assay, in which a scratch was introduced to a confluent monolayer of HRECs. Migrated cells into the area of the scratch were counted in response to differing concentrations of enantiopure homoisoflavanones (Figure 5). HREC migration was dose-dependently inhibited by the enantiopure homoisoflavanones (Figure 5). The (*S*)-isomers of **2** and **3** showed more potent effects than their (*R*)-forms. Similarly, the 3-benzyl-chromane (*R*)-**10**, which is the same conformation as the (*S*)-homoisoflavanones, had stronger inhibitory activity than the enantiomer (*S*)-**10**. However, (*R*)-**1** was more potent than (*S*)-**1** in this system.

The Matrigel tube formation assay requires most of the events of angiogenesis in vivo to be replicated, including migration, proliferation, and cell-cell adhesion. All enantiopure homoisoflavanones, with the exception of **2,** reduced tubule length in a dose-dependent fashion (Figure 6). Again, the (*S*)-isomer of homoisoflavanone derivative **3** had more

favorable activity than the (*R*)-isomer, with the conformationally similar (*R*)-**10** again showing stronger inhibitory activity than (*S*)-**10**.

CONCLUSION

We have developed asymmetric synthesis for the antiangiogenic natural product cremastranone and its homoisoflavanone derivatives. For this, asymmetric transfer hydrogenation of racemic 5,6,7-substituted-homoisoflavanones using Noyori's ruthenium catalysts afforded the enantiopure 3-benzyl-chroman-4-ol derivatives with excellent yield and enantioselectivity, although the C5-methoxy on the A ring affected the reactivity in the asymmetric transfer hydrogenation. By TPAP oxidation, (*R*)-3-benzyl-chroman-4-ol was oxidized smoothly into (*R*)-3-benzyl-chroman-4-one without racemization. During the enantioselective synthesis of cremastranone, a benzyl group was utilized as a protecting group for asymmetric transfer hydrogenation and alcohol oxidation. Two stereoisomers of homoisoflavanones synthesized were evaluated for their antiproliferative activity against HRECs and HUVECs, and the activity of the (*S*)-isomer was generally more effective than that of the (*R*)-form and racemate.. Interestingly, the absolute configuration of the natural product cremastranone was confirmed as the (*R*)-form, whereas the anti-angiogenic effect of the opposite (*S*)-homoisoflavanone was superior to the natural (*R*)-form in synthetic derivatives.

EXPERIMENTAL SECTION

Chemistry.

All starting materials and reagents were obtained from commercial sources and used without further purification. Air and moisture sensitive reactions were performed under nitrogen. Flash column chromatography was performed using silica gel 60(230–400 mesh, Merck) with the indicated solvents. Thin-layer chromatography (TLC) was performed using 0.25 mm silica gel plates (Merck). ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on a Bruker 600 MHz spectrometer as solution in deuterochloroform (CDCl$_3$) or methanol-d$_4$ (CD$_3$OD). ^{1}H NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and/or multiplet resonances), number of protons, and coupling constant (*J* value) in hertz (Hz). Enantiomeric excesses were determined by HPLC on an Agilent 1100 using one chiral column (Chiralpak AD-3 and IC-3). High resolution mass spectra (HRMS) were recorded on a JEOL JMS-700 (EI) and an Agilent 6530 Q-TOF LC/MS/MS system (ESI). The ECD spectra were acquired on an Applied Photophysics Chirascan-Plus circular dichroism spectrometer.

3-(3-Hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (2).

To a toluene (16 mL) solution of the 5,6,7-trimethoxychroman-4-one (**5a**) (1.0 g, 4.2 mmol) was added isovanillin (0.70 g, 4.6 mmol) and *p*-toluenesulfonic acid (82 mg, 0.43 mmol). The reaction mixture was refluxed for 12 h. After cooling to ambient temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the (*E*)-3-(3-hydroxy-4-methoxybenzylidene)-5,6,7-trimethoxychroman-4-one (0.96 g, 60%, BORSM

70%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.75 (s, 1H), 6.91 (d, 1H, J = 8.3 Hz), 6.88 (d, 1H, J = 2.0 Hz), 6.86 (t, 1H, J = 4.1 Hz), 6.26 (s, 1H), 5.69 (s, 1H), 5.25 (d, 2H, J = 1.7 Hz), 3.99 (s, 3H), 3.95 (s, 3H), 3.89 (s, 3H), 3.84 (s, 3H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 179.6, 171.2, 159.4, 159.2, 154.8, 147.5, 145.6, 136.3, 130.2, 128.2, 123.3, 115.8, 110.6, 96.2, 67.7, 61.7, 61.4, 60.4, 56.1, 56.0. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{20}$H$_{20}$O$_7$ Calcd for C$_{20}$H$_{20}$O$_7$ 372.1209; found 372.1208. A methanol solution of the (E)-3-(3-hydroxy-4-methoxybenzylidene)-5,6,7-trimethoxychroman-4-one (0.58 g, 1.6 mmol) and 10% Pd/C (82 mg) was placed under an atmosphere of hydrogen. After stirring for 20 min, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : n-hexane = 1 : 1) to afford the 3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**2**) (0.57 g, 97%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 6.82 (q, 2H, J = 4.8 Hz), 6.73 (q, 1H, J = 3.4 Hz), 6.27 (s, 1H), 5.63 (d, 1H, J = 1.9 Hz), 4.29 (q, 1H, J = 5.2 Hz), 4.12 (q, 1H, J = 6.3 Hz), 3.95 (s, 3H), 3.90 (d, 6H, J = 1.9 Hz), 3.84 (s, 3H), 3.20 (q, 1H, J = 6.1 Hz), 2.76 (m, 1H), 2.60 (q, 1H, J = 8.3 Hz). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 191.4, 159.8, 159.3, 154.5, 145.7, 145.3, 137.5, 131.7, 120.6, 115.3, 110.8, 108.0, 96.0, 69.1, 61.6, 61.3, 56.1, 56.0, 48.3, 32.2. HRMS (ESI) m/z: [M+H]$^+$ Calcd for C$_{20}$H$_{22}$O$_7$H 375.1444; found 375.1432.

7-Hydroxy-3-(3-hydroxy-4-methoxybenzyl)-5,6-dimethoxychroman-4-one (6).

To a toluene (3 mL) solution of the 7-benzyloxy-5,6-dimethoxychroman-4-one (**5b**) (0.27 g, 0.86 mmol) was added isovanillin (0.16 g, 0.99 mmol) and p-toluenesulfonic acid (19 mg, 0.10 mmol) at 0 °C. The reaction mixture was refluxed for 12 h. After cooling to ambient temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : n-hexane = 1 : 2) to afford the (E)-7-(benzyloxy)-3-(3-hydroxy-4-methoxybenzylidene)-5,6-dimethoxychroman-4-one (0.23 g, 59%, BORSM 76%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.74 (s, 1H), 7.44 (d, 2H, J = 7.1 Hz), 7.40 (t, 2H, J = 7.5 Hz), 7.34 (m, 1H), 6.90 (d, 1H, J = 8.3 Hz), 6.87 (d, 1H, J = 2.0 Hz), 6.84 (q, 1H, J = 3.5 Hz), 6.31 (s, 1H), 5.68 (s, 1H), 5.22 (d, 2H, J = 1.7 Hz), 5.14 (s, 2H), 4.00 (s, 3H), 3.94 (s, 3H), 3.85 (s, 3H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 179.6, 159.2, 158.3, 154.9, 147.5, 145.6, 138.2, 136.3, 135.9, 130.2, 128.7, 128.3, 128.2, 127.3, 123.3, 115.8, 110.9, 110.6, 97.5, 70.7, 67.7, 61.7, 61.4, 56.0. A methanol solution of the (E)-7-(benzyloxy)-3-(3-hydroxy-4-methoxybenzylidene)-5,6-dimethoxychroman-4-one (0.27 g, 0.60 mmol) and 10% Pd/C (64 mg) was placed under an atmosphere of hydrogen. After stirring for 45 min, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : n-hexane = 1 : 2) to afford the 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)-5,6-dimethoxychroman-4-one (**6**) (0.20 mg, 94%). ^{1}H NMR (600 MHz, CDCl$_3$) δ 6.80 (d, 2H, J = 2.0 Hz), 6.79 (d, 1H, J = 8.2 Hz), 6.71 (dd, 1H, J = 8.2 and 2.0 Hz), 6.33 (d, 1H, J = 5.8 Hz), 6.31 (s, 1H), 5.59 (s, 1H), 4.26 (dd, 1H, J = 11.3 and 4.1 Hz), 4.09 (dd, 1H, J = 11.3 and 7.6 Hz), 3.92 (s, 3H), 3.91 (s, 3H), 3.87 (s, 3H), 3.17 (dd, 1H, J = 14.4 and 4.4 Hz), 2.74 (m, 1H), 2.59 (dd, 1H, J = 14 and 10.7 Hz). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 191.6, 159.9, 155.6, 153.5, 145.7, 145.3, 135.2, 131.7, 120.6, 115.2, 110.8, 108.7, 98.9, 68.9, 61.6, 61.5, 56.0, 48.4, 32.2. HRMS (ESI) m/z: [M+H]$^+$ Calcd for C$_{19}$H$_{20}$O$_7$H 361.1287; found 361.1270.

3-(3-(Benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (7).

To an acetone (10 mL) solution of 3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**2**) (0.40 g, 1.1 mmol), benzyl bromide (0.2 mL, 1.7 mmol) and K_2CO_3 (0.3 g, 2.1 mmol) were added. The reaction mixture was refluxed for 4 h. After cooling to ambient temperature, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 3-(3-(benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**7**) (0.50 g, 99%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (d, 2H, *J* = 7.2 Hz), 7.36 (t, 2H, *J* = 7.3 Hz), 7.29 (t, 1H, *J* = 7.4 Hz), 6.84 (d, 1H, *J* = 7.9 Hz), 6.78 (d, 1H, *J* = 2 Hz), 6.76 (bs, 1H), 6.24 (s, 1H), 5.14 (s, 2H), 4.17 (dd, 1H, *J* = 11.3 and 4.1 Hz), 3.98 (dd, 1H, *J* = 11.4 and 7.5 Hz), 3.93 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.14 (dd, 1H, *J* = 13.8 and 4.1 Hz), 2.68 (m, 1H), 2.60 (dd, 1H, *J* = 13.8 and 10.7 Hz). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 191.4, 159.7, 159.3, 154.5, 148.5, 148.1, 137.5, 137.1, 130.9, 128.5, 127.9, 127.4, 121.9, 115.3, 111.9, 108.7, 95.9, 71.1, 68.9, 61.6, 61.3, 56.1, 56.1, 48.4, 32.3. HRMS (EI) m/z: $[M]^+$ Calcd for $C_{27}H_{28}O_7$ 464.1835; found 464.1837.

7-(Benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one (8).

[2 steps from **5b**] An anhydrous ethyl acetate solution of (*E*)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzylidene)-5,6-dimethoxychroman-4-one (88 mg, 0.16 mmol) and 10% Pd/C (8.7 mg) was placed under an atmosphere of hydrogen. After stirring for 30 min, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 4) to afford the 7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one (**8**) (49 mg, 56%, BORSM 97%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (m, 6H), 7.37 (m, 3H), 7.29 (t, 1H, *J* = 7.3 Hz), 6.84 (d, 1H, *J* = 7.9 Hz), 6.77 (d, 1H, *J* = 2 Hz), 6.76 (bs, 1H), 6.29 (s, 1H), 5.14 (s, 4H), 4.17 (dd, 1H, *J* = 11.3 and 4 Hz), 3.97 (dd, 1H, *J* = 11.3 and 7.5 Hz), 3.94 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 3.15 (dd, 1H, *J* = 13.7 and 4.1 Hz), 2.67 (m, 1H), 2.60 (dd, 1H, *J* = 13.8 and 10.7 Hz). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 191. 4, 159.5, 158.4, 154.6, 148.5, 148.1, 137.8, 137.1, 135.8, 130.9, 128.8, 128.5, 128.3, 127.9, 127.4, 127.3, 121.9, 115.3, 111.9, 108.9, 97.30, 71.1, 70.7, 68.9, 61.7, 61.4, 56.1, 48.4, 32.3. HRMS (EI) m/z: $[M]^+$ Calcd for $C_{33}H_{30}O_7$ 538.1992; found 538.1988. [2 steps from **6**] 1st step: To a *N,N*-dimethylformamide (2 mL) solution of 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)-5,6-dimethoxychroman-4-one (**6**) (22 mg, 0.061 mmol), benzyl bromide (22 μL, 0.18 mmol) and Cs_2CO_3 (60 mg, 0.18 mmol) were added. After stirring for 2 h at ambient temperature, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one (**8**) (24 mg, 72%).

cis-3-(3-Hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol (*cis*-9a).

To an anhydrous tetrahydrofuran (1 mL) solution of 3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**2**) (28 mg, 74 μmol), L-Selectride (0.32 mL, 0.15 mmol, 1 M in THF) was added dropwise at −78 °C. After stirring for 30 min, the reaction was quenched with NH_4Cl, extracted with ethyl ether, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (ethyl ether : *n*-hexane = 1 : 2) to afford the 3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol (*cis*-**9a**) (25 mg, 89%) . For *cis*-**9a**, ^1H NMR (600 MHz, $CDCl_3$) δ 6.84 (d, 1H, $J = 2.0$ Hz), 6.79 (d, 1H, $J = 8.2$ Hz), 6.74 (q, 1H, $J = 3.4$ Hz), 6.16 (s, 1H), 5.63 (d, 1H, $J = 5.0$ Hz), 4.69 (t, 1H, $J = 3.0$ Hz), 3.96 (s, 5H), 3.87 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 2.85 (q, 1H, $J = 7.2$ Hz), 2.58 (q, 1H, $J = 7.3$ Hz), 2.15 (m, 2H). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 153.4, 150.8, 149.8, 144.5, 144.0, 134.3, 131.7, 119.4, 114.2, 109.7, 109.5, 94.6, 63.9, 60.3, 59.9, 59.4, 54.9, 54.8, 39.0, 31.3. For *trans*-**9a**, ^1H NMR (600 MHz, $CDCl_3$) δ 6.78 (dd, 2H, $J = 5.3$ and 3.1 Hz), 6.66 (dd, 1H, $J = 8.2$ and 2.1 Hz), 6.23 (s, 1H), 5.57 (s, 1H), 4.59 (s, 1H), 4.11 (dd, 1H, $J = 10.9$ and 2.3 Hz), 3.99 (s, 3H), 3.95 (m, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 2.56 (dd, 1H, $J = 10.9$ and 7 Hz), 2.51 (d, 1H, $J = 2.5$ Hz), 2.45 (dd, 1H, $J = 13.9$ and 9.3 Hz), 2.18 (m, 1H). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 154.4, 153.0, 150.8, 146.1, 146.1, 135.7, 132.5, 119.8, 115.7, 111.4, 108.3, 95.6, 63.4, 61.0, 60.5, 60.0, 55.1, 54.9, 41.5, 33.5. HRMS (EI) m/z: $[M]^+$ Calcd for $C_{20}H_{24}O_7$ 376.1522; found 376.1519 for *cis*-**9a**; HRMS (EI) m/z: $[M]^+$ Calcd for $C_{20}H_{24}O_7$ 376.1522; found 376.1524 for *trans*-**9a**.

(*3R,4R*)-3-(3-Hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3R,4R*)-9a).

To an acetonitrile (1 mL) solution of 3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**2**) (21 mg, 55 μmol) and RuCl(*p*-cymene)[(*R,R*)-Ts-DPEN] (*R,R*)-**4** (10 mg, 16 μmol), an acetonitrile (1 mL) solution of DBU/formic acid (75uL : 25uL) was added at ambient temperature. After stirring for 24 h at 50 °C, the reaction was quenched with saturated aqueous NH_4Cl solution at ambient temperature. After extraction with diethyl ether, the organic layer was washed with an additional portion of saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford the (*3R,4R*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3R,4R*)-**9a**) (20 mg, 96%). $[\alpha]_D^{20} = +100$ (c 0.1, CH_2Cl_2). enantiomeric excess = 98%. ^1H NMR (600 MHz, $CDCl_3$) 6.85 (d, 1H, $J = 2.1$ Hz), 6.81 (d, 1H, $J = 8.2$ Hz), 6.75 (dd, 1H, $J = 8.2$ and 2.1 Hz), 6.16 (s, 1H), 5.59 (s, 1H), 4.69 (d, 1H, $J = 3.2$ Hz), 3.96 (s, 3H), 3.95 (m, 2H), 3.88 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 2.88 (dd, 1H, $J = 13.9$ and 7.7 Hz), 2.60 (dd, 1H, $J = 13.9$ and 7.9 Hz), 2.17 (m, 2H). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 153.4, 150.8, 149.8, 144.5, 144.0, 134.3, 131.7, 119.4, 114.2, 109.7, 109.5, 94.6, 63.9, 60.3, 59.9, 59.4, 54.9, 54.8, 39.0, 31.3. HRMS (EI) m/z: $[M]^+$ Calcd for $C_{20}H_{24}O_7$ 376.1522; found 376.1515. $T_R = 27$ min (chiral AD-3, *n*-hexane : EtOH = 60 : 40, flow rate = 0.5 mL/min).

(*3S,4S*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3S,4S*)-9a).

To an acetonitrile (1 mL) solution of 3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**2**) (20 mg, 53 μmol) and RuCl(*p*-cymene)[(*S,S*)-Ts-DPEN]

(*S,S*)-**4** (10 mg, 16 μmol), an acetonitrile (1 mL) solution of DBU/formic acid (75 μL : 25 μL) was added at ambient temperature. After stirring for 24 h at 50 °C, the reaction was quenched with saturated aqueous NH$_4$Cl solution at ambient temperature. After extraction with diethyl ether, the organic layer was washed with an additional portion of saturated aqueous NaHCO$_3$ solution, dried over MgSO$_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford the (*3S,4S*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3S,4S*)-**9a**) (18 mg, 92%). [α]$_D^{20}$ = −103 (c 0.1, CH$_2$Cl$_2$). enantiomeric excess = 97%. ^{1}H NMR (600 MHz, CDCl$_3$) 6.85 (d, 1H, *J* = 2.0 Hz), 6.80 (d, 1H, *J* = 8.2 Hz), 6.74 (dd, 1H, *J* = 8.2 and 2.1 Hz), 6.16 (s, 1H), 5.61 (s, 1H), 4.69 (d, 1H, *J* = 3.2 Hz), 3.97 (s, 5H), 3.88 (s, 3H), 3.80 (s, 3H), 3.78 (s, 3H), 2.88 (dd, 1H, *J* = 14 and 7.7 Hz), 2.60 (dd, 1H, *J* = 13.9 and 7.9 Hz), 2.15 (m, 2H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 153.4, 150.8, 149.8, 144.5, 144.0, 134.3, 131.7, 119.4, 114.2, 109.7, 109.5, 94.6, 63.9, 60.3, 59.9, 59.4, 54.9, 54.8, 39.0, 31.3. HRMS (EI) M=m/z: [M]$^+$ Calcd for C$_{20}$H$_{24}$O$_7$ 376.1522; found 376.1515. T$_R$ = 18 min (chiral AD-3, *n*-hexane : EtOH = 60 : 40, flow rate = 0.5 mL/min).

(*3R,4R*)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3R, 4R*)-9c).

To an acetonitrile (1 mL) solution of 3-(3-(benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**7**) (13 mg, 28 μmol) and (*R,R*)-**4** (5.2 mg, 8.2 μmol), an acetonitrile (1 mL) solution of DBU/formic acid (42 μL:14 μL) was added at ambient temperature. After stirring for 24 h at 50 °C, the reaction was quenched with saturated aqueous NH$_4$Cl solution at ambient temperature. After extraction with diethyl ether, the organic layer was washed with an additional portion of saturated aqueous NaHCO$_3$ solution, dried over MgSO$_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford the (*3R,4R*)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3R, 4R*)-**9c**) (12 mg, 93%). [α]$_D^{20}$ = + 60 (c 0.05, CH$_2$Cl$_2$). enantiomeric excess = 97%. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.44 (d, 2H, *J* = 7.1 Hz), 7.36 (t, 2H, *J* = 7.4 Hz), 7.29 (d, 1H, *J* = 7.4 Hz), 6.85 (d, 1H, *J* = 8.1 Hz), 6.82 (s, 1H), 6.81 (d, 2H, *J* = 7.7 Hz), 6.16 (s, 1H), 5.14 (s, 2H), 4.61 (s, 1H), 3.94 (s, 3H), 3.91 (d, 2H, *J* = 8.5 Hz), 3.87 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 2.83 (dd, 1H, *J* = 13.9 and 7.9 Hz), 2.57 (dd, 1H, *J* = 13.9 and 7.8 Hz), 2.11 (m, 1H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 154.4, 151.8, 150.8, 148.2, 148.0, 137.2, 135.3, 131.9, 128.5, 127.8, 127.4, 121.7, 115.4, 112.0, 110.5, 95.6, 71.1, 65.0, 61.3, 60.9, 60.3, 56.1, 55.9, 40.2, 32.4. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{27}$H$_{30}$O$_7$ 466.1992; found 466.1989. T$_R$ = 6.3 min (chiral AD-3, *n*-hexane : EtOH = 60 : 40, flow rate = 1.0 mL/min).

(*3S,4S*)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3S, 4S*)-9c).

To an acetonitrile (1 mL) solution of 3-(3-(benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one (**7**) (38 mg, 82 μmol) and (*S,S*)-**4** (15 mg, 24 μmol), an acetonitrile (1 mL) solution of DBU/formic acid (122 μL:40 μL) was added at ambient temperature. After stirring for 24 h at 50 °C, the reaction was quenched with saturated aqueous NH$_4$Cl solution at ambient temperature. After extraction with diethyl ether, the organic layer was washed with an additional portion of saturated aqueous NaHCO$_3$ solution, dried over MgSO$_4$, and concentrated under reduced pressure. The residue was purified by

flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford the (*3S*,*4S*)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3S*, *4S*)-**9c**) (33 mg, 87%). $[\alpha]_D^{20}$ = + 60 (c 0.05, CH$_2$Cl$_2$). enantiomeric excess = 93%. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.44 (d, 2H, *J* = 7.4 Hz), 7.36 (t, 2H, *J* = 7.4 Hz), 7.29 (d, 1H, *J* = 7.4 Hz), 6.85 (d, 1H, *J* = 8.0 Hz), 6.81 (d, 2H, *J* = 7.8 Hz), 6.16 (s, 1H), 5.14 (s, 2H), 4.61 (d, 1H, *J* = 3.4 Hz), 3.94 (s, 3H), 3.91 (d, 2H, *J* = 8.5 Hz), 3.87 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 2.83 (dd, 1H, *J* = 13.9 and 7.9 Hz), 2.57 (dd, 1H, *J* = 13.9 and 7.8 Hz), 2.11 (m, 1H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 154.4, 151.9, 150.8, 148.2, 148.0, 137.2, 135.3, 131.9, 128.5, 127.8, 127.4, 121.7, 115.4, 112.0, 110.5, 95.6, 71.1, 65.0, 61.3, 60.9, 60.3, 56.1, 55.9, 40.2, 32.4. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{27}$H$_{30}$O$_7$ 466.1992; found 466.1999. T$_R$ = 5.2 min (chiral AD-3, *n*-hexane : EtOH = 60 : 40, flow rate = 1.0 mL/min).

(*3R*,*4R*)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-ol ((*3R*, *4R*)-9d).

To an acetonitrile (1 mL) solution of 7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one (**8**) (16 mg, 31 μmol) and (*R*,*R*)-**4** (6.0 mg, 9.4 μmol), an acetonitrile (1 mL) solution of DBU/formic acid (46 μL:15 μL) was added at ambient temperature. After stirring for 24 h at 50 °C, the reaction was quenched with saturated aqueous NH$_4$Cl solution at ambient temperature. After extraction with diethyl ether, the organic layer was washed with an additional portion of saturated aqueous NaHCO$_3$ solution, dried over MgSO$_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford (*3R*,*4R*)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-ol ((*3R*,*4R*)-**9d**) (14 mg, 87%). enantiomeric excess = 98%. $[\alpha]_D^{20}$ = +56 (c 0.11, CH$_2$Cl$_2$). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.45 (dd, 4H, *J* = 9.7 and 7.9 Hz), 7.37 (dd, 4H, *J* = 17.8 and 7.4), 7.33 (t, 1H, *J* = 7.3 Hz), 7.29 (t, 1H, *J* = 7.4 Hz), 6.86 (d, 1H, *J* = 8.2 Hz), 6.82 (d, 2H, *J* = 7.2 Hz), 6.22 (s, 1H), 5.15 (s, 2H), 5.06 (s, 2H), 4.62 (s, 1H), 3.97 (s, 3H), 3.91 (m, 2H), 3.88 (s, 3H), 3.82 (s, 3H), 2.84 (dd, 1H, *J* = 13.9 and 7.9 Hz), 2.58 (dd, 1H, *J* = 13.8 and 7.7 Hz), 2.10 (m, 2H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 153.5, 151.9, 150.7, 148.2, 148.0, 137.2, 136.7, 135.7, 131.9, 128.6, 128.5, 127.9, 127.9, 127.4, 127.2, 121.7, 115.4, 112.0, 110.9, 97.3, 71.1, 70.6, 65.0, 61.4, 61.1, 60.3, 56.1, 40.1, 32.4. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{33}$H$_{34}$O$_7$ 542.2305; found 542.2303. T$_R$ = 15.6 min (chiral AD-3, *n*-hexane : EtOH = 60 : 40, flow rate = 1.0 mL/min).

(*3S*,*4S*)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-ol ((*3S*, *4S*)-9d).

To an acetonitrile (1 mL) solution of 7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one (**8**) (19 mg, 0.04 mmol) and (*S*,*S*)-**4** (6.5 mg, 0.01 mmol), an acetonitrile (1 mL) solution of DBU/formic acid (60 μL:20 μL) was added at ambient temperature. After stirring for 24 h at 50 °C, the reaction was quenched with saturated aqueous NH$_4$Cl solution at ambient temperature. After extraction with diethyl ether, the organic layer was washed with an additional portion of saturated aqueous NaHCO$_3$ solution, dried over MgSO$_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford the (*3S*,*4S*)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-

dimethoxychroman-4-ol ((*3S,4S*)-**9d**) (17 mg, 92%). $[\alpha]_D^{20} = -53$ (c 0.15, CH_2Cl_2). enantiomeric excess = 96%. ^1H NMR (600 MHz, $CDCl_3$) δ 7.45 (dd, 4H, *J* = 10.4 and 7.9 Hz), 7.39 (dd, 4H, *J* = 17.6 and 7.3 Hz), 7.33 (t, 1H, *J* = 7.3 Hz), 7.30 (t, 1H, *J* = 7.4 Hz), 6.86 (d, 1H, *J* = 7.9 Hz), 6.82 (d, 2H, *J* = 8.0 Hz), 6.22 (s, 1H), 5.15 (s, 2H), 5.06 (s, 2H), 4.62 (s, 1H), 3.97 (s, 3H), 3.92 (m, 2H), 3.88 (s, 3H), 3.82 (s, 3H), 2.84 (dd, 1H, *J* = 13.9 and 7.9 Hz), 2.58 (dd, 1H, *J* = 13.9 and 7.7 Hz), 2.13m, 1H). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 153.5, 151.9, 150.7, 148.2, 148.0, 137.2, 136.7, 135.7, 131.9, 128.6, 128.5, 127.9, 127.9, 127.4, 127.2, 121.7, 115.4, 112.0, 110.9, 97.3, 71.1, 70.6, 65.0, 61.4, 61.1, 60.3, 56.1, 40.1, 32.4. HRMS (EI) m/z: $[M]^+$ Calcd for $C_{33}H_{34}O_7$ 542.2305; found 542.2302. T_R = 10 min (chiral AD-3, *n*-hexane : EtOH = 60 : 40, flow rate = 1.0 mL/min).

(*R*)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((*R*)-7).

To a dichloromethane (2 mL) solution of (*3R,4R*)-**9c** (21 mg, 42 μmol), tetrapropylammonium perruthenate (4.5 mg, 12 μmol), molecular sieves (4 Å, 50 mg) and *N*-methylmorpholine *N*-oxide (7.5 mg, 64 μmol) were added. After stirring for 1 h at ambient temperature. the reaction mixture was filtered through a Celite pad with CH_2Cl_2 as eluent, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3 to 1 : 1) to afford the (*R*)-3-[(3-benzyloxy-4-methoxy-phenyl)methyl]-5,6,7-trimethoxy-chroman-4-one ((*R*)-**7**) (20 mg, 66%). $[\alpha]_D^{20} = -25$ (c 0.12, CH_2Cl_2). enantiomeric excess = 94%. ^1H NMR (600 MHz, $CDCl_3$) δ 7.45 (d, 2H, *J* = 7.1 Hz), 7.37 (t, 2H, *J* = 7.3 Hz), 7.30 (t, 1H, *J* = 7.3 Hz), 6.83 (d, 1H, *J* = 7.9 Hz), 6.78 (d, 1H, *J* = 1.9 Hz), 6.76 (s, 1H), 6.24 (s, 1H), 5.14 (s, 2H), 4.19 (dd, 1H, *J* = 11.3 and 4.1 Hz), 3.97 (dd, 1H, *J* = 11.3 and 7.4 Hz), 3.93 (s, 3H), 3.89 (s, 3H), 3.87 (s, 3H), 3.81 (s, 3H), 3.15 (dd, 1H, *J* = 13.8 and 4.1 Hz), 2.66 (m, 1H), 2.60 (dd, 1H, *J* = 13.8 and 10.7 Hz). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 191.4, 159.7, 159.3, 154.5, 148.5, 148.1, 137.5, 137.1, 130.9, 128.5, 127.9, 127.5, 121.9, 115.3, 111.9, 108.7, 95.9, 71.1, 68.9, 61.6, 61.3, 56.1, 56.1, 48.4, 32.3. HRMS (EI) m/z: $[M]^+$ Calcd for $C_{27}H_{28}O_7$ 464.1835; found 464.1834. T_R = 4.7 min (chiral AD-3, ACN : MeOH = 50 : 50, flow rate = 0.5 mL/min).

(*R*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((*R*)-2).

An methanol solution of the (*R*)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((*R*)-**7**) (11 mg, 24 μmol) and 10% Pd/C (2.6 mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 1) to afford the (*R*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((*R*)-**2**) (8.0 mg, 88%). $[\alpha]_D^{20} = -37$ (c 0.11, CH_2Cl_2). enantiomeric excess = 96%. ^1H NMR (600 MHz, $CDCl_3$) δ 6.80 (d, 1H, *J* = 2 Hz), 6.80 (d, 1H, *J* = 8.2 Hz), 6.71 (dd, 1H, *J* = 8.2 and 2.0 Hz), 6.25 (s, 1H), 5.60 (s, 1H), 4.28 (dd, 1H, *J* = 11.3 and 4.2 Hz), 4.10 (dd, 1H, *J* = 11.3 and 7.7 Hz), 3.93 (s, 3H), 3.88 (d, 6H, *J* = 2.6 Hz), 3.82 (s, 3H), 3.19 (dd, 1H, *J* = 14 and 4.3 Hz), 2.76 (m, 1H), 2.60 (dd, 1H, *J* = 14 and 10.9 Hz). $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 191.4, 159.7, 159.3, 154.5, 145.7, 145.3, 137.5, 131.7, 120.6, 115.2, 110.8, 108.7, 96.0, 69.1, 61.6, 61.3, 56.1, 56.0, 48.4, 32.2. HRMS (EI) m/z: $[M]^+$ Calcd for $C_{20}H_{22}O_7$ 374.1366; found 374.1366. T_R = 5.0 min (chiral AD-3, ACN : MeOH = 50 : 50, flow rate = 1.0 mL/min). For (*S*)-**2**, $[\alpha]_D^{20} = +40$ (c 0.10,

CH$_2$Cl$_2$). enantiomeric excess = 99%. T$_R$ = 5.0 min (chiral AD-3, ACN : MeOH = 50 : 50, flow rate = 0.5 mL/min).

2-Methoxy-5-(((R)-5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl(*tert*-butoxycarbonyl)-L-phenylalaninate ((*R*,*S*)-3).

To a dichloromethane solution (1 mL) of (*R*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((*R*)-2) (13 mg, 33 μmol) were added Boc-L-Phe-OH (11 mg, 42 μmol), EDCI (10 mg, 52 μmol) and DMAP (1 mg, 8.2 μmol). After stirring for 17 h, the reaction mixture was diluted with dichloromethane and washed with water and brine, dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 2-methoxy-5-(((*R*)-5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl(*tert*-butoxycarbonyl)-D-phenylalaninate ((*R*,*S*)-3) (17 mg, 80%). [α]$_D^{20}$ = – 28 (c 0.17, CH$_2$Cl$_2$). enantiomeric excess = >99%. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.35 (t, 2H, *J* = 7.5 Hz), 7.30 (t, 3H, *J* = 7 Hz), 7.18 (d, 1H, *J* = 6.5 Hz), 7.09 (dd, 1H, *J* = 8.3 and 1.9 Hz), 6.92 (d, 1H, *J* = 8.4 Hz), 6.84 (s, 1H), 6.25 (s, 1H), 5.03 (d, 1H, *J* = 8.4 Hz), 4.86 (dd, 1H, *J* = 14.2 and 6.2 Hz), 4.30 (dd, 1H, *J* = 11.3 and 4.1 Hz), 4.10 (dd, 1H, *J* = 11.3 and 7.6 Hz), 3.93 (s, 3H), 3.89 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.35 (dd, 1H, *J* = 14.0 and 5.7 Hz), 3.24 (dd, 1H, *J* = 14 and 6.4 Hz), 3.20 (dd, 1H, *J* = 14 and 4.3 Hz), 2.74 (m, 1H), 2.65 (dd, 1H, *J* = 13.9 and 10.7 Hz), 1.42 (s, 9H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 191.1, 170.1, 159.7, 159.4, 155.1, 154.5, 149.7, 139.3, 137.5, 136.0, 131.1, 129.6, 128.6, 127.6, 127.1, 123.4, 112.7, 108.7, 96.0, 80.0, 68.9, 61.6, 61.3, 56.1, 55.9, 54.4, 48.3, 38.3, 31.8, 28.3. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{34}$H$_{39}$NO$_{10}$ 621.2574; found 621.2573. T$_R$ = 171.9 min (chiral IC, IC-3, TFA in DW : ACN = 50 : 50, flow rate = 0.7 mL/min).

2-Methoxy-5-(((R)-5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl(*tert*-butoxycarbonyl)-D-phenylalaninate ((*R*,*R*)-3).

To a dichloromethane solution (1 mL) of (*R*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((*R*)-2) (22 mg, 59 μmol) were added Boc-D-Phe-OH (19 mg, 72 μmol), EDCI (17 mg, 89 μmol) and DMAP (1.5 mg, 12 μmol). After stirring for 17 h, the reaction mixture was diluted with dichloromethane and washed with water and brine, dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 2-methoxy-5-(((*R*)-5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl(*tert*-butoxycarbonyl)-D-phenylalaninate ((*R*,*R*)-3) (27 mg, 73%). [α]$_D^{20}$ = – 20 (c 0.27, CH$_2$Cl$_2$). enantiomeric excess = 99%. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.33 (m, 5H), 7.08 (d, 1H, *J* = 7.7 Hz), 6.92 (d, 1H, *J* = 8.1 Hz), 6.84 (s, 1H), 6.26 (s, 1H), 5.03 (bs, 1H), 4.87 (d, 1H, *J* = 5.6 Hz), 4.28 (d, 1H, *J* = 8.2 Hz), 4.10 (m, 1H), 3.93 (s, 3H), 3.88 (s, 3H), 3.81 (s, 3H), 3.81 (s, 3H), 3.34 (dd, 1H, *J* = 13.1 and 4.3 Hz), 3.22 (m, 2H), 2.72 (bs, 1H), 2.65 (t, 1H, *J* = 12.8 Hz), 1.42 (s, 9H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 191.1, 170.1, 159.7, 159.4, 155.1, 154.5, 149.7, 139.3, 137.5, 136.0, 131.1, 129.6, 128.6, 127.6, 127.1, 123.4, 112.7, 108.7, 96.0, 80.0, 68.9, 61.6, 61.3, 56.1, 55.9, 54.4, 48.3, 38.3, 31.8, 28.3. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{34}$H$_{39}$NO$_{10}$ 621.2574; found 621.2574. T$_R$ = 138.8 min (chiral IC, IC-3, TFA in DW : ACN = 50 : 50, flow rate = 0.7 mL/min).

2-Methoxy-5-(((S)-5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl(*tert*-butoxycarbonyl)-L-phenylalaninate ((S,S)-3).

To a dichloromethane solution (1 mL) of (S)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((S)-**2**) (10 mg, 28 μmol) were added Boc-L-Phe-OH (9 mg, 34 μmol), EDCI (8 mg, 42 μmol) and DMAP (0.6 mg, 4.9 μmol). After stirring for 17 h, the reaction mixture was diluted with dichloromethane and washed with water and brine, dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 2-methoxy-5-(((S)-5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl(tert-butoxycarbonyl)-L-phenylalaninate ((**S,S**)-**3**) (15 mg, 86%). [α]$_D^{20}$ = + 20 (c 0.15, CH$_2$Cl$_2$). enantiomeric excess = 99%. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.35 (t, 2H, *J* = 7.4 Hz), 7.30 (t, 3H, *J* = 6.9 Hz), 7.08 (dd, 1H, *J* = 8.3 and 1.7 Hz), 6.92 (d, 1H, *J* = 8.3 Hz), 6.84 (s, 1H), 6.25 (s, 1H), 5.03 (d, 1H, *J* = 6.8 Hz), 4.87 (d, 1H, *J* = 7.7 Hz), 4.29 (dd, 1H, *J* = 11.3 and 4.1 Hz), 4.10 (dd, 1H, *J* = 11.3 and 7.6 Hz), 3.93 (s, 3H), 3.88 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.35 (dd, 1H, *J* = 14.0 and 5.7 Hz), 3.23 (dd, 1H, *J* = 13.9 and 6.4 Hz), 3.20 (dd, 1H, *J* = 14 and 4.3 Hz), 2.74 (m, 1H), 2.64 (dd, 1H, *J* = 13.8 and 10.7 Hz), 1.42 (s, 9H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 191.1, 170.1, 159.7, 159.4, 155.1, 154.5, 149.7, 139.3, 137.5, 136.0, 131.1, 129.6, 128.6, 127.6, 127.1, 123.4, 112.7, 108.7, 96.0, 80.0, 68.9, 61.6, 61.3, 56.1, 55.9, 54.4, 48.3, 38.3, 31.8, 28.3. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{34}$H$_{39}$NO$_{10}$ 621.2574; found 621.2572. T$_R$ = 161.1 min (chiral IC, IC-3, TFA in DW : ACN = 50 : 50, flow rate = 0.7 mL/min).

2-Methoxy-5-(((S)-5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl(*tert*-butoxycarbonyl)-D-phenylalaninate ((S,R)-3).

To a dichloromethane solution (1 mL) of (S)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((S)-**2**) (14 mg, 37 μmol) were added Boc-D-Phe-OH (12 mg, 45 μmol), EDCI (11 mg, 57 μmol) and DMAP (1 mg, 8.2 μmol). After stirring for 17 h, the reaction mixture was diluted with dichloromethane and washed with water and brine, dried over MgSO$_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the 2-methoxy-5-(((S)-5,6,7-trimethoxy-4-oxochroman-3-yl)methyl)phenyl(tert-butoxycarbonyl)-D-phenylalaninate ((**S,R**)-**3**) (21 mg, 90%). [α]$_D^{20}$ = + 29. (c 0.21, CH$_2$Cl$_2$). enantiomeric excess = 97%. ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.35 (t, 2H, *J* = 7.5 Hz), 7.30 (t, 3H, *J* = 7 Hz), 7.09 (dd, 1H, *J* = 8.3 and 1.8 Hz), 6.93 (d, 1H, *J* = 8.4 Hz), 6.84 (s, 1H), 6.25 (s, 1H), 5.03 (d, 1H, *J* = 6.7 Hz), 4.88 (d, 1H, *J* = 7.9 Hz), 4.29 (dd, 1H, *J* = 11.3 and 4.1 Hz), 4.10 (dd, 1H, *J* = 11.3 and 7.4 Hz), 3.94 (s, 3H), 3.88 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.36 (dd, 1H, *J* = 13.9 and 5.6 Hz), 3.24 (dd, 1H, *J* = 13.9 and 6.4 Hz), 3.20 (dd, 1H, *J* = 14.1 and 4.3 Hz), 2.75 (m, 1H), 2.65 (dd, 1H, *J* = 13.9 and 10.7 Hz), 1.43 (s, 9H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 191.1, 170.1, 159.7, 159.4, 155.1, 154.5, 149.7, 139.3, 137.5, 136.0, 131.1, 129.6, 128.6, 127.6, 127.1, 123.4, 112.7, 108.7, 96.0, 80.0, 68.9, 61.6, 61.3, 56.1, 55.9, 54.4, 48.3, 38.3, 31.8, 28.3. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{34}$H$_{39}$NO$_{10}$ 621.2574; found 621.2574. T$_R$ = 130.0 min (chiral IC, IC-3, TFA in DW : ACN = 50 : 50, flow rate = 0.7 mL/min).

(*S*)-2-methoxy-5-((5,6,7-trimethoxychroman-3-yl)methyl)phenol ((*S*)-10).

[With catalytic hydrogenation] A methanol solution of (*R*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((*R*)-**2**) (19 mg, 50 μmol) and 10% Pd/C (5.3 mg) was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the (*S*)-2-methoxy-5-((5,6,7-trimethoxychroman-3-yl)methyl)phenol ((*S*)-**10**) (14 mg, 78%). $[\alpha]_D^{20}$ = + 28 (c 0.14, CH_2Cl_2). enantiomeric excess = 71%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.78 (q, 2H, *J* = 2.7 Hz), 6.66 (q, 1H, *J* = 3.4 Hz), 6.18 (s, 1H), 5.58 (d, 1H, *J* = 2.2 Hz), 4.08 (m, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.79 (s, 6H), 3.72 (q, 1H, *J* = 6.3 Hz), 2.75 (m, 1H), 2.57 (t, 2H, *J* = 8.0 Hz), 2.32 (q, 1H, *J* = 8.3 Hz), 2.20 (m, 1H). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 152.2, 151.6, 150.7, 145.5, 145.1, 135.8, 132.7, 120.4, 115.1, 110.6, 107.3, 95.9, 69.7, 61.0, 60.6, 56.0, 55.9, 37.6, 33.6, 25.5. [With Et_3SiH] To a tetrahydrofuran solution of trifluoroacetic acid (0.16 mL) and triethylsilane (32 μL, 0.2 mmol) was added anhydrous tetrahydrofuran (0.55 mL) solution of (*3R,4R*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3R,4R*)-**9a**) (16 mg, 43 μmol) dropwise over 20 min. After stirring for 1 h at 40 °C, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and NaHCO_3 and dried over MgSO_4. The solvent was removed under reduced pressure and purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 4) to afford the (*S*)-2-methoxy-5-((5,6,7-trimethoxychroman-3-yl)methyl)phenol ((*S*)-**10**) (7.8 mg, 50%). $[\alpha]_D^{20}$ = + 55 (c 0.055, CH_2Cl_2). enantiomeric excess = 99%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.79 (d, 1H, *J* = 4.1 Hz), 6.78 (d, 1H, *J* = 1.9 Hz) 6.67 (dd, 1H, *J* = 8.2 and 2 Hz), 6.18 (s, 1H), 5.59 (s, 1H), 4.08 (qd, 1H, *J* = 10.6 and 1.6 Hz), 3.88 (s, 3H), 3.85 (s, 3H), 3.80 (s, 6H), 3.74 (dd, 1H, *J* = 10.5 and 8.3 Hz), 2.75 (ddd, 1H, *J* = 16.4, 5.5 and 1.3 Hz), 2.61 (sep, 2H, *J* = 7.3 Hz), 2.32 (dd, 1H, *J* = 16.4 and 8.3 Hz), 2.23 (m, 1H). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 152.3, 151.8, 150.8, 145.7, 145.2, 135.9, 132.9, 120.6, 115.3, 110.8, 107.4, 96.1, 69.8, 61.2, 60.7, 56.1, 55.9, 37.7, 33.8, 25.6. HRMS (EI) m/z: [M]+ Calcd for $C_{20}H_{24}O_6$ 360.1573; found 360.1576. T_R = 5.5 min (chiral AD-3, *n*-hexane : EtOH = 60 : 40, flow rate = 1.0 mL/min).

(*R*)-2-methoxy-5-((5,6,7-trimethoxychroman-3-yl)methyl)phenol ((*R*)-10).

[With catalytic hydrogenation] A solution of (*S*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-one ((*S*)-**2**) (20 mg, 0.052 mmol) and 10% Pd/C (5.6 mg) in methanol was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the (*R*)-2-methoxy-5-((5,6,7-trimethoxychroman-3-yl)methyl)phenol ((*R*)-**10**) (15 mg, 78.4%). $[\alpha]_D^{20}$ = − 34 (c 0.15, CH_2Cl_2). enantiomeric excess = 78%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.78 (q, 2H, *J* = 2.7 Hz), 6.67 (d, 1H, *J* = 2.0 Hz), 6.18 (s, 2H), 5.60 (s, 1H), 4.08 (m, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.79 (s, 6H), 3.72 (q, 1H, *J* = 6.3 Hz), 2.75 (m, 1H), 2.57 (t, 1H, *J* = 8.0 Hz), 2.32 (q, 1H, *J* = 8.3 Hz), 2.20 (m, 1H). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 152.2, 151.6, 150.7, 145.5, 145.1, 135.8, 132.7, 120.4, 115.1, 110.6, 107.3, 95.9, 69.6, 61.0, 60.6, 56.0, 55.9, 37.6, 33.6, 25.5. [With Et_3SiH]

To a 40 °C mixture of trifluoroacetic acid (0.15 mL) and triethylsilane (32 μL, 0.2 mmol) was added anhydrous tetrahydrofuran (0.5 mL) solution of (*3S,4S*)-3-(3-hydroxy-4-methoxybenzyl)-5,6,7-trimethoxychroman-4-ol ((*3S,4S*)-**9a**) (15 mg, 40 μmol) dropwise over 20 min. After stirring for 1 h, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and NaHCO$_3$ and dried over MgSO$_4$. The solvent was removed under reduced pressure and purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 4) to afford the (*R*)-2-methoxy-5-((5,6,7-trimethoxychroman-3-yl)methyl)phenol ((*R*)-**10**) (6.2 mg, 43%). $[\alpha]_D^{20} = -32$ (c 0.062, CH$_2$Cl$_2$). enantiomeric excess = 98%. ^{1}H NMR (600 MHz, CDCl$_3$) δ 6.79 (d, 1H, *J* = 4.4 Hz), 6.78 (d, 1H, *J* =1.6 Hz) 6.67 (dd, 1H, *J* = 8.2 and 2 Hz), 6.18 (s, 1H), 5.60 (s, 1H), 4.09 (qd, 1H, *J* = 10.6 and 1.6 Hz), 3.88 (s, 3H), 3.85 (s, 3H), 3.79 (s, 6H), 3.72 (dd, 1H, *J* = 10.4 and 8.3 Hz), 2.77 (ddd, 1H, *J* = 16.5, 5.5 and 1.3 Hz), 2.61 (sep, 2H, *J* = 7.3 Hz), 2.34 (dd, 1H, *J* = 16.5 and 8.4 Hz), 2.22 (m, 1H). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 152.3, 151.8, 150.8, 145.7, 145.2, 135.9, 132.9, 120.6, 115.3, 110.8, 107.4, 96.1, 69.8, 61.2, 60.7, 56.1, 55.9, 37.7, 33.8, 25.6.. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{20}$H$_{24}$O$_6$ 360.1573; found 360.1572. T$_R$ = 7.6 min (chiral AD-3, *n*-hexane : EtOH = 60 : 40, flow rate = 1.0 mL/min).

Racemic 2-methoxy-5-((5,6,7-trimethoxychroman-3-yl)methyl)phenol (rac-10).

Racemic 3-benzyl-chromane was prepared by catalytic hydrogenation of 3-benzylidene-chroman-4-one using hydrogen and Pd/C. Particularly, the anhydrous methanol solution of (*E*)-3-(3-hydroxy-4-methoxybenzylidene)-5,6,7-trimethoxychroman-4-one (22 mg, 59 μmol) and 10% Pd/C (6 mg) was placed under an atmosphere of hydrogen. After stirring for 24 h, the reaction mixture was diluted with ethyl acetate, filtered through a Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 2) to afford the rac-**10** (19 mg, 88%).

(*R*)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one ((*R*)-11).

To a dichloromethane (3 mL) solution of (*3R,4R*)-**9d** (11mg, 20 μmol), tetrapropylammonium perruthenate (4 mg, 10 μmol), molecular sieves (4 Å, 8 mg) and *N*-methylmorpholine *N*-oxide (8 mg, 70 μmol) were added. After stirring for 1 h at ambient temperature. the reaction mixture was filtered through a Celite pad with dichloromethane as eluent, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford the (*R*)-**11** (10 mg, 94%). $[\alpha]_D^{20} = -18$ (c 0.056, CH$_2$Cl$_2$). ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.44 (d, 4H, *J* = 7.3 Hz), 7.42 (t, 2H, *J* = 7.3 Hz), 7.37 (t, 3H, *J* = 7.3 Hz), 7.30 (t, 1H, *J* = 7.3 Hz), 6.84 (d, 1H, *J* = 7.9 Hz), 6.77 (d, 2H, *J* = 8.2 Hz), 6.30 (s, 1H), 5.14 (s, 4H), 4.17 (dd, 1H, *J* = 11.3 and 4.1 Hz), 3.98 (dd, 1H, *J* = 11.3 and 7.6 Hz), 3.94 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 3.14 (dd, 1H, *J* = 13.8 and 4.1 Hz), 2.68 (m, 1H), 2.60 (dd, 1H, *J* = 13.8 and 10.7 Hz). ^{13}C{^{1}H} NMR (150 MHz, CDCl$_3$) δ 191.4, 159.6, 158.4, 154.6, 148.5, 148.1, 137.8, 137.1, 135.8, 130.9, 128.8, 128.5, 128.3, 127.9, 127.5, 127.3, 121.9, 115.3, 112.0, 108.9, 97.3, 71.1, 70.7, 68.9, 61.7, 61.4, 56.1, 48.4, 32.3. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{33}$H$_{32}$O$_7$ 540.2148; found 540.2153. T$_R$ = 19.1 min (chiral AD-3, *n*-hexane : EtOH = 60 : 40, flow rate = 1.0 mL/min).

(S)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one ((S)-11).

To a dichloromethane (3 mL) solution of (3S,4S)-**9d** (15 mg, 7.6 μmol), tetrapropylammonium perruthenate (5 mg, 14 μmol), molecular sieves (4 Å, 8 mg) and *N*-methylmorpholine *N*-oxide (10 mg, 90 μmol) were added. After stirring for 1 h at ambient temperature. The reaction mixture was filtered through a Celite pad with dichloromethane as eluent, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford the (S)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one ((S)-**11**) (11 mg, 72%). $[\alpha]_D^{20}$ = + 20 (c 0.050, CH_2Cl_2). enantiomeric excess = 94%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (d, 4H, *J* = 7.4 Hz), 7.42 (t, 2H, *J* = 7.3 Hz), 7.37 (t, 3H, *J* = 7.3 Hz), 7.30 (t, 1H, *J* = 7.3 Hz), 6.84 (d, 1H, *J* = 7.9 Hz), 6.77 (d, 2H, *J* = 9.1 Hz), 6.29 (s, 1H), 5.14 (s, 4H), 4.15 (dd, 1H, *J* = 11.3 and 4.0 Hz), 3.98 (dd, 1H, *J* = 11.3 and 4 Hz), 3.94 (s, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 3.14 (dd, 1H, *J* = 13.8 and 4.0 Hz), 2.68 (m, 1H), 2.60 (dd, 1H, *J* = 13.7 and 10.9 Hz). ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 191.4, 159.6, 158.4, 154.6, 148.5, 148.1, 137.8, 137.1, 135.8, 130.9, 128.8, 128.5, 128.3, 127.9, 127.5, 127.3, 121.9, 115.3, 112.0, 108.9, 97.3, 71.1, 70.7, 68.9, 61.7, 61.4, 56.1, 48.4, 32.3. HRMS (EI) m/z: [M]$^+$ Calcd for $C_{33}H_{32}O_7$ 540.2148; found 540.2153. T_R = 10.8 min (chiral AD-3, ACN : MeOH = 50 : 50, flow rate = 0.5 mL/min).

(R)-Cremastranone ((R)-1).

To a dichloromethane (2 mL) solution of (R)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one ((R)-**11**) (8.5 mg, 13 μmol) was added boron trichloride (70 μL, 0.07 mmol, 1 M CH_2Cl_2) at −20 °C. After stirring for 10 min, the reaction mixture was diluted with dichloromethane and washed with $NaHCO_3$ and water, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford the (R)-Cremastranone ((R)-**1**) (4.5 mg, 99%). $[\alpha]_D^{20}$ = − 29 (c 0.034, MeOH). enantiomeric excess = 93%. ^{1}H NMR (600 MHz, CD_3OD) δ 6.86 (d, 1H, *J* = 8.2 Hz), 6.71 (d, 1H, *J* = 1.9 Hz), 6.68 (d, 1H, *J* = 8.2 Hz), 5.92 (s, 1H), 4.24 (dd, 1H, *J* = 11.4 and 4.3 Hz), 4.08 (dd, 1H, *J* = 11.3 and 7.4 Hz), 3.83 (s, 3H), 3.78 (s, 3H), 3.09 (dd, 1H, *J* = 13.9 and 4.5 Hz), 2.83 (m, 1H), 2.64 (dd, 1H, *J* = 13.8 and 10.3 Hz). ^{13}C{^{1}H} NMR (150 MHz, CD_3OD) δ 198.7, 159.4, 158.7, 155.5, 146.5, 146.3, 130.9, 129.0, 120.0, 115.6, 111.5, 101.6, 94.4, 68.9, 59.6, 55.0, 46.6, 31.7. HRMS (EI) m/z: [M]$^+$ Calcd for $C_{18}H_{18}O_7$ 346.1053; found 346.1057. T_R = 11.8 min (chiral AD-3, ACN : MeOH = 50 : 50, flow rate = 0.5 mL/min).

(S)-Cremastranone ((S)-1).

To a dichloromethane (2 mL) solution of (S)-7-(benzyloxy)-3-(3-(benzyloxy)-4-methoxybenzyl)-5,6-dimethoxychroman-4-one ((S)-**11**) (8.5 mg, 13 μmol) was added boron trichloride (70 μL, 0.07 mmol, 1 M CH_2Cl_2) at − 20 °C. After stirring for 10 min, the reaction mixture was diluted with dichloromethane and washed with $NaHCO_3$ and water, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate : *n*-hexane = 1 : 3) to afford the (S)-Cremastranone ((S)-**1**) (4.5 mg, 99%). $[\alpha]_D^{20}$ = + 18 (c 0.06, CH_3OH). enantiomeric excess = 80%. ^{1}H NMR (600 MHz, CD_3OD) δ 6.85 (d, 1H, *J* = 8.2 Hz), 6.70 (s, 1H), 6.66

(d, 1H, J = 8.2 Hz), 5.90 (s, 1H), 4.23 (qd, 1H, J = 11.4, 4.3 and 2 Hz), 4.06 (m, 1H), 3.82 (s, 3H), 3.77 (s, 3H), 3.07 (dd, 1H, J = 13.9 and 4.5 Hz), 2.81 (m, 1H), 2.63 (dd, 1H, J = 13.7 and 10.8 Hz). ^{13}C{^{1}H} NMR (150 MHz, CD$_3$OD) δ 198.7, 159.3, 158.7, 155.5, 146.5, 146.3, 130.9, 129.0, 120.0, 115.6, 111.5, 101.6, 94.4, 68.9, 59.6, 55.0, 46.6, 31.7. HRMS (EI) m/z: [M]$^+$ Calcd for C$_{18}$H$_{18}$O$_7$ 346.1053; found 346.1058. T_R = 8.4 min (chiral AD-3, ACN : MeOH = 50 : 50, flow rate = 0.5 mL/min).

Biological assay materials.

Human retinal microvascular endothelial cells (HRECs; Cell Systems, Seattle, WA) and human umbilical vein endothelial cells (HUVECs; Lonza, Walkersville, MD), were used between passage 5 and 8. Endothelial Growth Medium (EGM-2) was made by adding an EGM-2 "Bullet Kit" (Cat no. CC-4147) to Endothelial Basal Medium (EBM) (Cat no. CC-3156) (Lonza). 92–1 uveal melanoma cells (a kind gift of Dr. Martine Jager, University of Leiden) were grown in RPMI medium containing 10% FBS and 1% penicillin-streptomycin (pen-strep). Y-79 retinoblastoma cells (a kind gift of Dr. Brenda L. Gallie, Hospital for Sick Children) were grown in RB medium (IMDM + 10% FBS + 55 μM β-mercaptoethanol + 10 μg/mL insulin + 1% pen-strep). AlamarBlue (product code BUF012B) was from AbD Serotec (Raleigh, NC), while Matrigel matrix basement membrane (Cat no. 354234) was from Corning (Corning, NY).

Cell proliferation assay.

These compounds' ability to affect cell proliferation was assessed by an alamarBlue fluorescence assay as previously described.[19] Four cell types were used: HRECs, HUVECs, 92–1, and Y79. Briefly, 2,500 cells in 100 μL growth medium were seeded in 96-well clear bottom black plates and grown for 24 hours at 37°C, 5% CO$_2$ Each test compound was then added (concentration range: 0.1 nM to 500 μM) followed by 44 hours' incubation at 37°C, 5% CO$_2$ (DMSO final concentration = 1%). Then, alamarBlue reagent (11.1 μL) was added and after 4 hours, fluorescence was read on a Synergy H1 plate reader (Biotek, Winooski, VT) with excitation and emission wavelengths of 560 nm and 590 nm, respectively. GraphPad Prism software (v. 7.0) was used for analysis, fitting data to three-parameter log(dose) vs. response curves. Compounds that reduced cell number by 50% at the highest concentration tested (relative to DMSO control) were reported as having a GI$_{50}$ < 100 μM.

In vitro migration assay.

HREC migration was monitored as described before,[14,15] with modifications. Briefly, HRECs were grown until confluency in 96-well plates. A scratch was made across the center of each well with a sterile 10-μL micropipette tip, and fresh complete medium containing DMSO or indicated concentrations of compounds were added to the wells (DMSO final concentration = 1%). Wells were imaged at 4x using an IncuCyte ZOOM system (Essen BioScience, Ann Arbor, MI) with images taken every 2 hours to monitor cell migration across the scratch. After 8 hours, the cells that migrated into the scratch were counted using the Multipoint tool in ImageJ.

In vitro tube formation assay.

A Matrigel tube formation assay was used to test the ability of HRECs to form tube-like structures when treated with different concentrations of compounds according to our standard protocol.[19] Briefly, 15,000 cells in 100 μL EGM-2 medium were grown at 37°C, 5% CO_2 in the absence (DMSO treated) and presence of different concentrations of compounds in 96-well clear plates coated with 50 μL of Matrigel basement membrane (DMSO final concentration = 1%). After 8 hours, images were recorded using an EVOS FL microscope and tubule length was measured using Angiogenesis Analyzer macros in ImageJ (http://image.bio.methods.free.fr/ImageJ/?Angiogenesis-Analyzer-for-ImageJ).

Statistical analyses.

Data from tube formation and migration experiments were analyzed by one-way ANOVA with Dunnett's post hoc tests for comparisons between compound treatments and DMSO control. All analyses were performed using GraphPad Prism software (v.7.0). A p value of <0.05 was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

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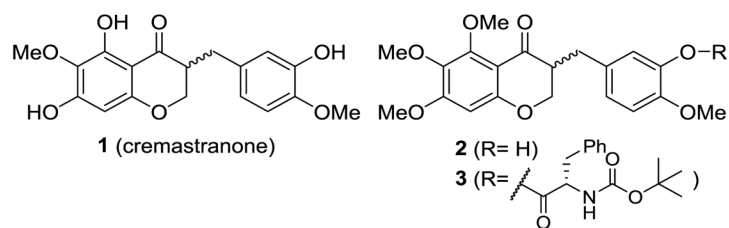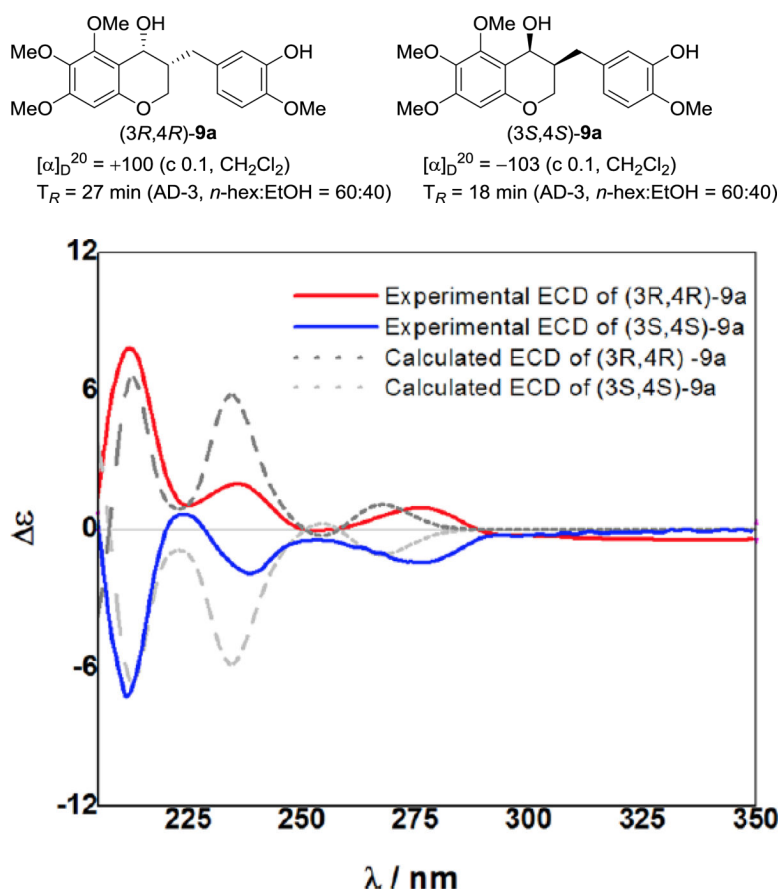
compound[a]	HREC	HUVEC	92-1	Y79
1 (natural)	ND[b]	1.5	ND	ND
1 (racemic)	0.22	0.38	9.8	47
2	2	12	>100	>100
3	0.055	0.75	>100	12

Figure 1.
Anti-angiogenic activity of natural and synthetic homoisoflavanones. HREC, target human retinal microvascular endothelial cells; HUVEC, human umbilical vein endothelial cells; 92–1, uveal melanoma cell line; and Y79, retinoblastoma cell line, used as surrogates for non-target ocular cell types. [a]see references 12 and 14a, [b]ND: not determined
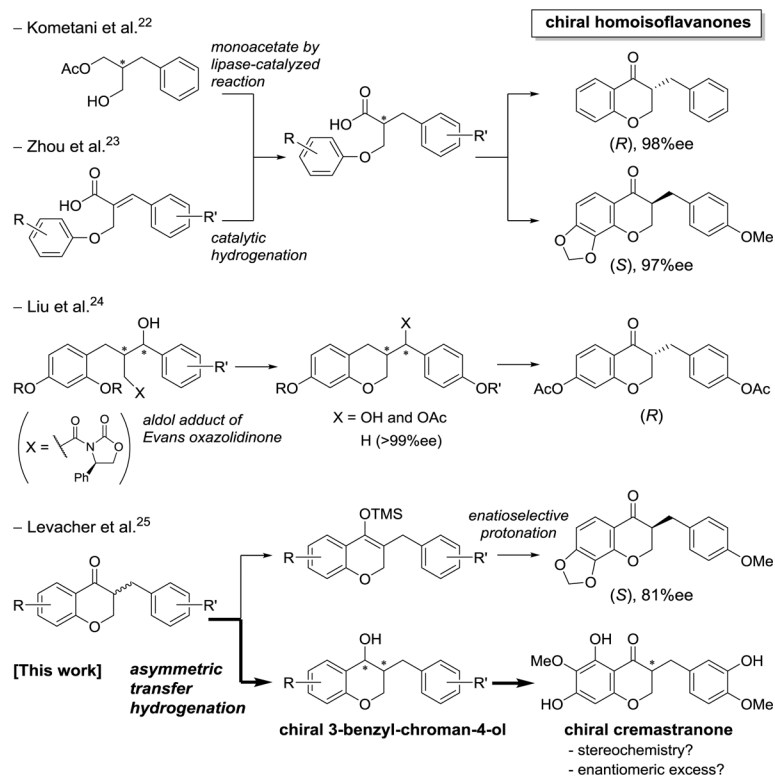
Figure 2.
Previous approaches and our plan for asymmetric synthesis of homoisoflavanones

Figure 3.
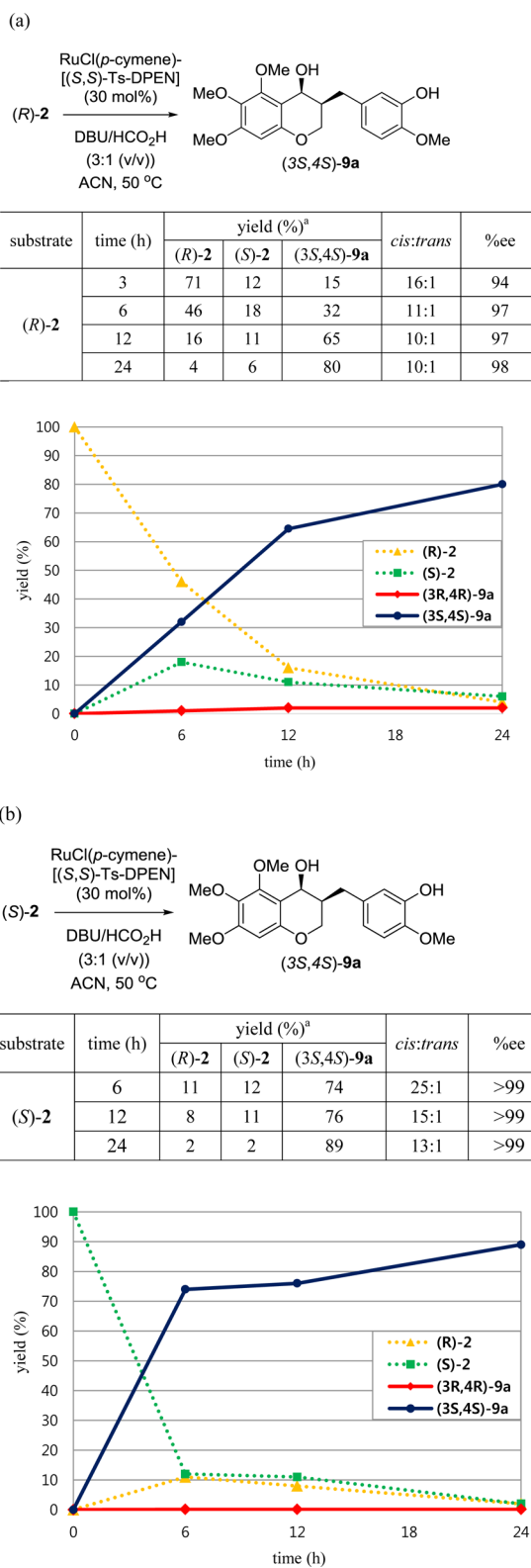Experimental and calculated ECD spectra of (3*R*,4*R*)-**9a** and (3*S*,4*S*)-**9a**

(a)

substrate	time (h)	yield (%)[a]			cis:trans	%ee
		(R)-2	(S)-2	(3S,4S)-9a		
(R)-2	3	71	12	15	16:1	94
	6	46	18	32	11:1	97
	12	16	11	65	10:1	97
	24	4	6	80	10:1	98

(b)

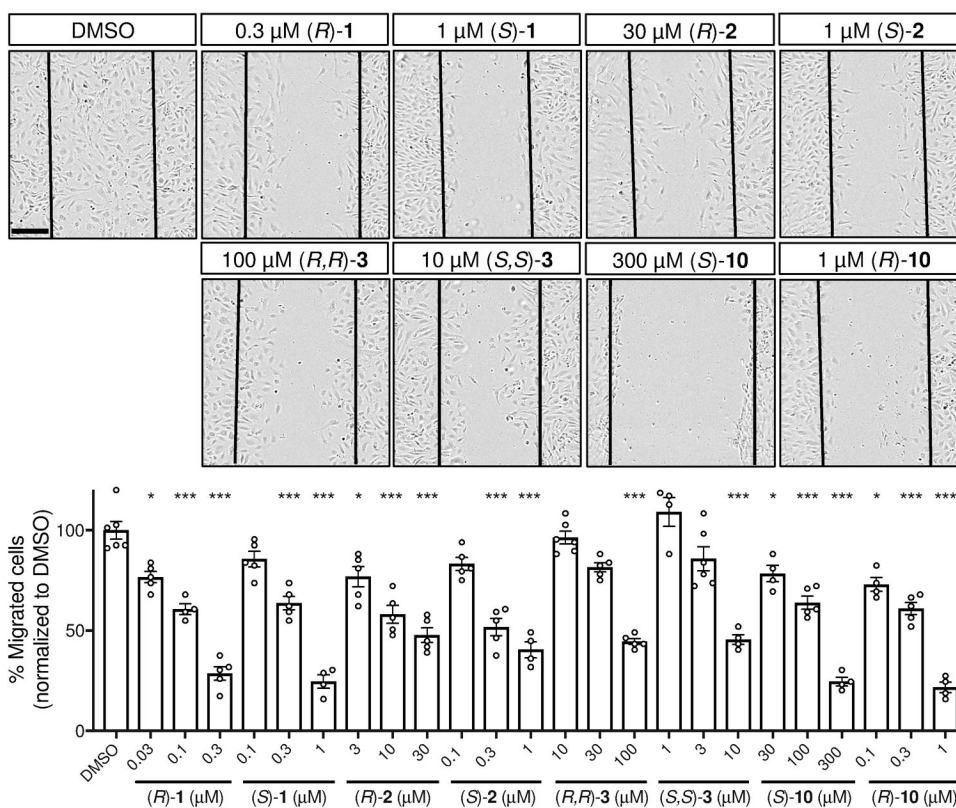| substrate	time (h)	yield (%)[a]			cis:trans	%ee
		(R)-2	(S)-2	(3S,4S)-9a		
(S)-2	6	11	12	74	25:1	>99
	12	8	11	76	15:1	>99
	24	2	2	89	13:1	>99

Figure 4.

Matched/mismatched effects on a pair of homoisoflavanones and catalyst (*S,S*)-**4** in asymmetric transfer hydrogenation. (a) (*R*)-**2**; (b) (*S*)-**2**. [a] The concentration of each compound was determined by chiral HPLC analysis using a Chiralpak AD-3.
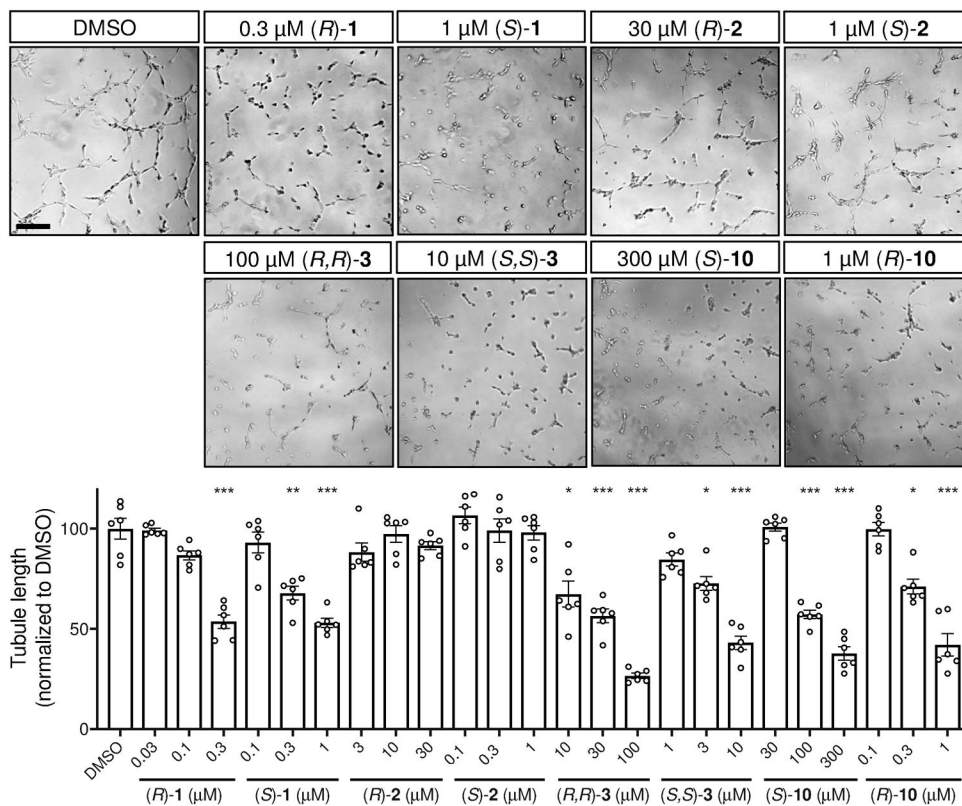
Figure 5.
Effects of potent enantiomers on HREC migration. Inhibition of HREC migration in a scratch-wound assay. Top, images of control and highest dose treatments, with time zero scratch position marked. Scale bar = 200 μm. Bottom, quantification of migration. Dose ranges chosen based on GI_{50} data. Mean±SEM, n=4–6; *p<0.05, ***p<0.001, ANOVA with Dunnett's post hoc tests. Representative data from three independent experiments.

Figure 6.
Effects of potent enantiomers on HREC tube formation. Inhibition of HREC tube formation in a Matrigel tubule-formation assay Top, images of control and highest dose treatments. Scale bar = 200 μm. Bottom, quantification of tubule length. Dose ranges chosen based on GI$_{50}$ data. Mean±SEM, n=6; *p<0.05, **p<0.01, ***p<0.001, ANOVA with Dunnett's post hoc tests. Representative data from three independent experiments ((R)-**1** and (S)-**1** were assessed in a separate experiment but combined here for ease of visualization).
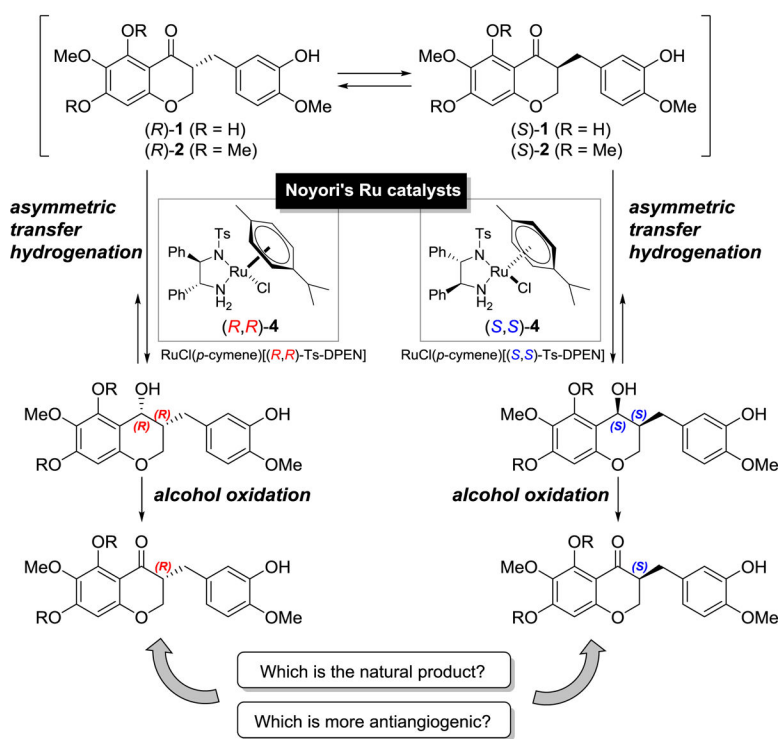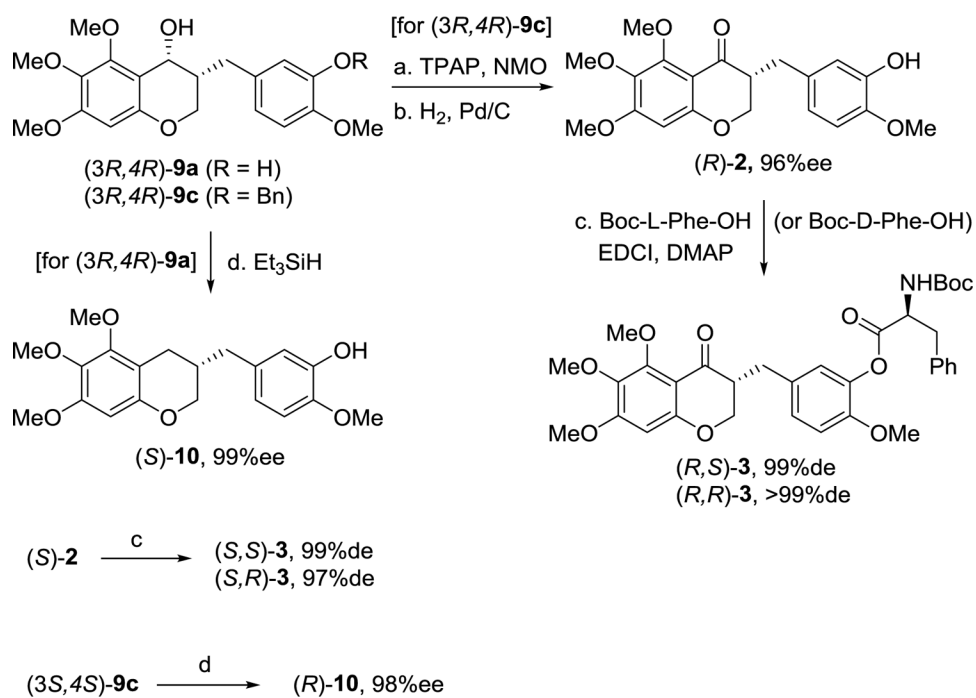
Scheme 1.
Enantioselective synthesis of cremastranone (**1**) and its analog (**2**) via asymmetric transfer hydrogenation using Noyori's ruthenium catalysts **4a** and **4b**.
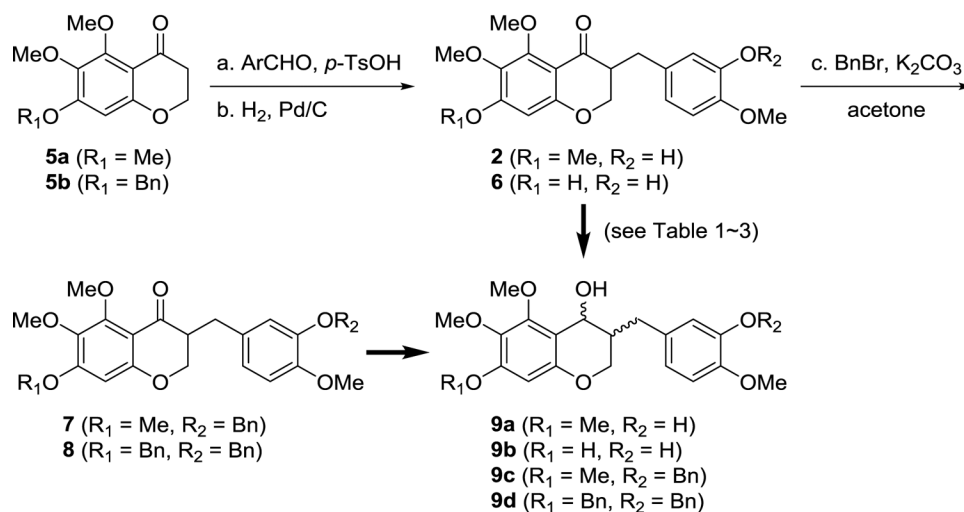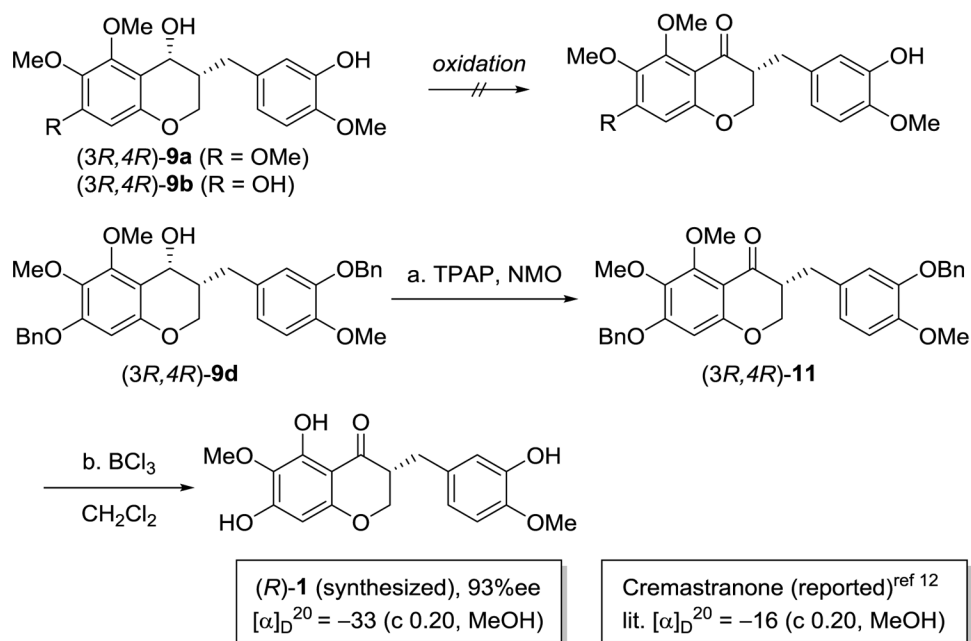
Scheme 2.
Preparation of racemic homoisoflavanones for the synthesis of 3-benzyl-chroman-4-ol.

Reagents and conditions: (a) isovanillin or 3-benzyloxy-4-methoxybenzaldehyde, *p*-TsOH, benzene, reflux, 70% for **2**; 76% for **6** and **8**; (b) H₂, Pd/C, MeOH, rt, 98% for **2**; 97% for **6**; 56% (97% BORSM) for **8**; (c) benzyl bromide, K₂CO₃, acetone, 99% for **7** and 72% for **8**.

Scheme 3.

Synthesis of chiral 3-benzyl-chroman-4-one and 3-benzyl-chromane derivatives.

Reagents and conditions: (a) TPAP, NMO, MS 4 Å, CH$_2$Cl$_2$, 66%; (b) H$_2$, Pd/C, EtOAc, 88%; (c) Boc-L-Phe-OH or Boc-D-Phe-OH, EDCI, DMAP, CH$_2$Cl$_2$, 80% for (*R,S*)-**3** and 73% for (*R,R*)-**3**; (d) Et$_3$SiH, TFA, THF, 40 °C, 51%.

Scheme 4.
Asymmetric synthesis of cremastranone.

Reagents and conditions: (a) TPAP, NMO, MS 4 Å, CH$_2$Cl$_2$, 1 h, 94%; (b) BCl$_3$, CH$_2$Cl$_2$, −20 °C, 10 min, 99%.
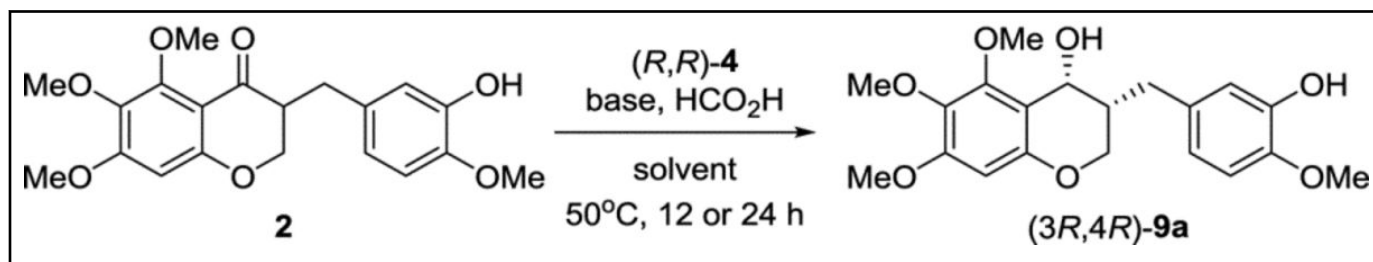
Table 1.

C4-Carbonyl reduction of homoisoflavanones **2** and **7**.

entry	substrate	reducing agent	temp (°C)	yield (%)	cis : trans[a]
1	**2**	NaBH$_4$	0	68	3.3 : 1
2	**7**	NaBH$_4$, CeCl$_3$	0	85	3.5 : 1
3	**7**	LiAlH$_4$	0	81	1.3 : 1
4	**7**	DIBAL	−40	98	2.2 : 1
5	**7**	Super-H®	0	72	3.2 : 1
6	**7**	Super-H®	−78	92	3.4 : 1
7	**7**	L-selectride	0	61	3.8 : 1
8	**2**	L-selectride	−78	89	>20 : 1
9	**7**	L-selectride	−78	99	>20 : 1

[a]The ratio of *cis*- to *trans*-isomers was assessed by ^{1}H NMR spectroscopy

Table 2.

Asymmetric transfer hydrogenation of homoisoflavanone **2** using Noyori's ruthenium catalysts.

entry	catalyst (mol%)	base (equiv.)	base:HCO$_2$H (v/v)	solvent	yield (%)^c	cis:trans	cis ee (%)^e
1^a	(R,R)-**4** (10)	Et$_3$N (3.5)	1:3	EtOAc	16 (32)	>20:1	ND^d
2	(R,R)-**4** (10)	Et$_3$N (3.5)	1:3	EtOAc	38 (51)	>20:1	98
3	(R,R)-**4** (10)	Et$_3$N (3.5)	1:3	ACN	NR	-	-
4	(R,R)-**4** (10)	Et$_3$N (10)	3:1	EtOAc	45 (80)	>20:1	ND
5^b	(S,S)-**4** (10)	Et$_3$N (10)	3:1	EtOAc	56 (92)	>20:1	85
6	(R,R)-**4** (20)	Et$_3$N (10)	3:1	ACN	25 (64)	ND^d	ND
7	(R,R)-**4** (20)	DBU (10)	3:1	EtOAc	33 (35)	ND	97
8	(R,R)-**4** (20)	DBU (10)	3:1	ACN	23 (26)	ND	97
9	(R,R)-**4** (30)	DBU (10)	3:1	EtOAc	91	ND	97
10	(R,R)-**4** (30)	DBU (10)	3:1	ACN	96	>20:1	97
11^b	(S,S)-**4** (30)	DBU (10)	3:1	ACN	92	>20:1	97
12	(R,R)-**4** (10)	DBU (5.0)	10:1	ACN	35 (54)	1:1	97

^{a}Without degassing;

^{b}major product is (3S,4S)-**9a**;

^{c}recovered yield based on starting material in parentheses; NR: no reaction;

^{d}ND: not determined;

^{e}the enantiomeric excess (%ee) was determined by chiral HPLC analysis using a Chiralpak AD-3.

Table 3.

Screening conditions for carbonyl reduction of benzyl-protected homoisoflavonoids by Noyori's chiral ruthenium catalysts.

6 (R$_1$ = H, R$_2$ = H)
7 (R$_1$ = Me, R$_2$ = Bn)
8 (R$_1$ = Bn, R$_2$ = Bn)

(3R,4R)-9b
(3R,4R)-9c
(3R,4R)-9d

entry	substrate	catalyst (mol%)	base (equiv.)	solvent	yield (%)	*cis:trans*	*cis* ee (%)[d]
1[a]	6	(R,R)-4 (10)	Et$_3$N (10)	EtOAc	trace	-	-
2	6	(R,R)-4 (30)	DBU (10)	ACN	trace	-	-
3[a]	7	(R,R)-4 (10)	Et$_3$N (10)	EtOAc	32	>20:1	90
4	7	(R,R)-4 (10)	DBU (10)	ACN	55	ND[c]	99
5	7	(R,R)-4 (30)	DBU (10)	EtOAc	73	>20:1	ND
6	7	(R,R)-4 (30)	DBU (10)	ACN	93	ND	97
7	7	(S,S)-4 (30)	DBU (10)	ACN	92	>20:1	93
8	8	(R,R)-4 (30)	DBU (10)	ACN	87	>20:1	98
9	8	(S,S)-4 (30)	DBU (10)	ACN	92	>20:1	96

[a] stirred for 12 h;

[b] major product is (3S,4S)-9a;

[c] ND: not determined;

[d] the enantiomeric excess (%ee) was determined by chiral HPLC analysis using a Chiralpak AD-3.

Table 4.

Growth inhibitory activity (GI$_{50}$, μM) of racemic and enantiopure homoisoflavanones (95% confidence interval shown in parentheses)

compound	structure	HREC	HUVEC	92–1	Y79
rac-**1**		0.22 (0.12 – 0.39)	0.38 (0.24 – 0.59)	48 (17 – 132)	9.8 (2.1 – 45)
(*R*)-**1**		2.0 (1.0 – 3.8)	0.98 (0.30 – 3.2)	4.5 (1.6 – 12)	3.9 (1.8 – 8.5)
(*S*)-**1**		0.35 (0.087 – 1.2)	0.24 (0.16 – 0.36)	0.34 (0.15 – 0.72)	0.36 (0.14 – 0.89)
rac-**2**		2.0 (0.81 – 5.1)	12 (2.7 – 55)	>100	>100
(*R*)-**2**		0.44 (0.21 – 0.94)	0.55 (0.16 – 1.9)	>100	0.40 (0.013 – 1.3)
(*S*)-**2**		0.024 (0.0078 – 0.074)	0.0027 (0.00066 – 0.011)	>100	0.014 (0.012 – 0.017)
rac-**3**		0.055 (0.032 – 0.094)	0.75 (0.37 – 1.5)	>100	12 (5.7 – 25)
(*R,S*)-**3**		0.27 (0.088 – 0.85)	0.0039 (0.0011 – 0.013)	>100	0.030 (0.0087 – 0.11)
(*S,S*)-**3**		0.028 (0.0087 – 0.093)	0.0030 (0.00067 – 0.013)	>100	0.03 (0.011 – 0.082)
(*R,R*)-**3**		4.0 (1.3 – 13)	1.1 (0.73 – 1.6)	>100	2.5 (1.2 – 5.2)
(*S,R*)-**3**		0.16 (0.087 – 0.31)	0.16 (0.040 – 0.64)	>100	0.22 (0.044 – 1.1)
cis-**9a**		0.034 (0.0081 – 0.15)	1.1 (0.56 – 2.4)	>100	>100
trans-**9a**		>100	3.9 (0.17 – 130)	>100	>100

(3*R*,4*R*)-**9a**		0.72 (0.41 – 1.2)	0.35 (0.087 – 1.4)	>100	0.42 (0.091 – 1.9)
(3*S*,4*S*)-**9a**		0.23 (0.049 – 0.95)	0.036 (0.0067 – 0.19)	>100	0.070 (0.038 – 0.13)
rac-**10**		0.0015 (0.00056 – 0.0041)	0.0062 (0.00015 – 0.30)	28 (3.1 – 246)	17 (11 – 27)
(*S*)-**10**		2.0 (0.52 – 7.3)	1.7 (0.41 – 6.9)	>100	3.3 (1.0 – 11)
(*R*)-**10**		0.0065 (0.0046 – 0.0094)	0.0093 (0.0015 – 0.056)	>100	0.015 (0.0022 – 0.11)